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(54) Title: B7-LIKE MOLECULES AND USES THEREOF

(57) Abstract: The present invention provides B7-Like(B7-L) polypeptides and nucleic acid molecules encoding the same. The invention also provides selective binding agents, vectors, host cells, and methods for producing B7-L polypeptides. The invention further provides pharmaceutical compositions and methods for the diagnosis, treatment, amelioration, and/or prevention of diseases, disorders, and conditions associated with B7-L polypeptides.

B7-LIKE MOLECULES AND USES THEREOF

This application claims the benefit of priority from U.S. Provisional Patent Application No. 60/215,645, filed on June 30, 2000, the disclosure of which is 5 explicitly incorporated by reference herein.

Field of the Invention

The present invention relates to B7-Like (B7-L) polypeptides and nucleic acid molecules encoding the same. The invention also relates to selective binding agents, 10 vectors, host cells, and methods for producing B7-L polypeptides. The invention further relates to pharmaceutical compositions and methods for the diagnosis, treatment, amelioration, and/or prevention of diseases, disorders, and conditions associated with B7-L polypeptides.

15 Background of the Invention

Technical advances in the identification, cloning, expression, and manipulation of nucleic acid molecules and the deciphering of the human genome have greatly accelerated the discovery of novel therapeutics. Rapid nucleic acid sequencing techniques can now generate sequence information at unprecedented rates 20 and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of polypeptide-encoding regions. A comparison of a predicted amino acid sequence against a database compilation of known amino acid sequences allows one to determine the extent of homology to previously identified sequences and/or structural landmarks. 25 The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product for structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides may confer advantageous properties on a product for use as a therapeutic.

In spite of the significant technical advances in genome research over the past 30 decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics or those encoding polypeptides, which may act as "targets" for therapeutic molecules, have still not been identified. Accordingly, it is an

object of the invention to identify novel polypeptides, and nucleic acid molecules encoding the same, which have diagnostic or therapeutic benefit.

Summary of the Invention

5 The present invention relates to novel B7-L nucleic acid molecules and encoded polypeptides.

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence as set forth in SEQ ID NO: 1;
- 10 (b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2;
- (c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of either (b) or (c); and
- (d) a nucleotide sequence complementary to either (b) or (c).

15

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide which is at least about 70 percent identical to the polypeptide as set forth in SEQ ID NO: 2, wherein the 20 encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1 or (a);
- 25 (c) a region of the nucleotide sequence of SEQ ID NO: 1, (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide fragment has an activity of the polypeptide set forth in SEQ ID NO: 2, or is antigenic;
- (d) a region of the nucleotide sequence of SEQ ID NO: 1 or any of (a)-
- (e) comprising a fragment of at least about 16 nucleotides;
- 30 (f) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d); and
- (g) a nucleotide sequence complementary to any of (a)-(d).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 5 (c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 10 (d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 15 (e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (f) a nucleotide sequence of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;
- 20 (g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (f); and
- (h) a nucleotide sequence complementary to any of (a) - (e).

The present invention provides for an isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2.

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence as set forth in SEQ ID NO: 3, optionally further comprising an amino-terminal methionine;
- 30 (b) an amino acid sequence for an ortholog of SEQ ID NO: 2;
- (c) an amino acid sequence which is at least about 70 percent identical to the amino acid sequence of SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;

- (d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 comprising at least about 25 amino acid residues, wherein the fragment has an activity of the polypeptide set forth in SEQ ID NO: 2, or is antigenic; and
5 (e) an amino acid sequence for an allelic variant or splice variant of the amino acid sequence as set forth in SEQ ID NO: 2 or any of (a) -(c).

The invention further provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- 10 (a) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
15 (b) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
20 (c) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
25 (d) the amino acid sequence as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2; and
25 (e) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2.

The invention still further provides for an isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution selected from the group consisting of: methionine at position 4; leucine or methionine at position 12; leucine or valine at position 13; leucine or valine at position 16; leucine or valine at position 17; leucine, valine, or methionine at position 18; leucine or valine at position 23; leucine at position 26; leucine or valine at position 27; valine or leucine at position 31; leucine or isoleucine at position 39; alanine at position 46; valine at position 48; alanine at position 52; isoleucine at

position 54; glutamic acid at position 61; valine at position 64; glutamic acid at position 66; leucine, methionine, or valine at position 67; valine or leucine at position 69; arginine at position 73; leucine at position 76; valine at position 79; methionine at position 80; tyrosine at position 83; arginine at position 84; aspartic acid at position 85; arginine at position 87; glutamic acid at position 88; aspartic acid at position 92; tyrosine at position 97; lysine at position 98; leucine, isoleucine, or methionine at position 103; isoleucine, leucine, or methionine at position 108; isoleucine at position 115; isoleucine at position 117; leucine or isoleucine at position 120; valine or isoleucine at position 122; serine at position 123; glutamic acid at position 124; serine at position 127; phenylalanine at position 128; arginine at position 129; phenylalanine at position 131; valine at position 132; alanine at position 137; valine or isoleucine at position 143; alanine at position 148; glycine at position 149; isoleucine or methionine at position 155; isoleucine or methionine at position 157; isoleucine at position 166; tyrosine at position 174; isoleucine, leucine, or methionine at position 179; isoleucine at position 180; leucine at position 194; phenylalanine at position 215; serine at position 218; serine at position 222; isoleucine or leucine at position 226; valine or leucine at position 227; leucine or valine at position 231; isoleucine at position 240; aspartic acid at position 242; methionine or leucine at position 245; arginine at position 246; threonine at position 256; valine or isoleucine at position 260; leucine or isoleucine at position 262; leucine or valine at position 268; valine or methionine at position 272; valine at position 273; valine, isoleucine, or methionine at position 275; phenylalanine at position 278; valine or isoleucine at position 279; isoleucine or valine at position 281; and arginine at position 282; wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2.

25

Also provided are fusion polypeptides comprising B7-L amino acid sequences.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising the recombinant nucleic acid molecules as set forth herein, and a method of producing a B7-L polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced.

A transgenic non-human animal comprising a nucleic acid molecule encoding a B7-L polypeptide is also encompassed by the invention. The B7-L nucleic acid

molecules are introduced into the animal in a manner that allows expression and increased levels of a B7-L polypeptide, which may include increased circulating levels. Alternatively, the B7-L nucleic acid molecules are introduced into the animal in a manner that prevents expression of endogenous B7-L polypeptide (*i.e.*, generates 5 a transgenic animal possessing a B7-L polypeptide gene knockout). The transgenic non-human animal is preferably a mammal, and more preferably a rodent, such as a rat or a mouse.

Also provided are derivatives of the B7-L polypeptides of the present invention.

10 Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the B7-L polypeptides of the invention. Such antibodies and peptides may be agonistic or antagonistic.

15 Pharmaceutical compositions comprising the nucleotides, polypeptides, or selective binding agents of the invention and one or more pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules, and selective binding agents.

20 The B7-L polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, and/or detect diseases and disorders, including those recited herein.

25 The present invention also provides a method of assaying test molecules to identify a test molecule that binds to a B7-L polypeptide. The method comprises contacting a B7-L polypeptide with a test molecule to determine the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of a B7-L polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of B7-L polypeptide or on the activity of B7-L polypeptide.

30 Methods of regulating expression and modulating (*i.e.*, increasing or decreasing) levels of a B7-L polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding a B7-L polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of a B7-L polypeptide may be administered.

Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described herein.

In another aspect of the present invention, the B7-L polypeptides may be used for identifying receptors thereof ("B7-L polypeptide receptors"). Various forms of "expression cloning" have been extensively used to clone receptors for protein ligands. See, e.g., Simonsen and Lodish, 1994, *Trends Pharmacol. Sci.* 15:437-41 and Tartaglia et al., 1995, *Cell* 83:1263-71. The isolation of a B7-L polypeptide receptor is useful for identifying or developing novel agonists and antagonists of the B7-L polypeptide signaling pathway. Such agonists and antagonists include soluble B7-L polypeptide receptors, anti-B7-L polypeptide receptor-selective binding agents (such as antibodies and derivatives thereof), small molecules, and antisense oligonucleotides, any of which can be used for treating one or more disease or disorder, including those disclosed herein.

15 Brief Description of the Figures

Figures 1A-1C illustrate the nucleotide sequence of the human B7-L gene (SEQ ID NO: 1) and the deduced amino acid sequence of human B7-L polypeptide (SEQ ID NO: 2). The predicted signal peptide is indicated (underline);

20 Figures 2A-2C illustrate an amino acid sequence alignment of human B7-L polypeptide (Agp1_51578; SEQ ID NO: 2), human CD80 or B7-1 (Cd80_Human; SEQ ID NO: 10; GenBank accession no. P33681), human CD86 or B7-2 (Cd86_Human; SEQ ID NO: 11; GenBank accession no. U04343), human B7-H1 (B7-H1_Human; SEQ ID NO: 12; GenBank accession no. AF177937), human B7rp-1 (B7rp-1_Human; SEQ ID NO: 13; GenBank accession no. AF199028), human PRO352 (Pro352_Human; SEQ ID NO: 14; GenBank accession no. Y41705), human butyrophilin BTF1 (Btf1_Human; SEQ ID NO: 15; GenBank accession no. U90543), human butyrophilin BTF2 (Btsf2a2_Hu; SEQ ID NO: 16; GenBank accession no. U90550), human butyrophilin BTF4 (Btf4_Human; SEQ ID NO: 17; GenBank accession no. U90546), human butyrophilin BTF3 (Btn3a3_Human; SEQ ID NO: 18; GenBank accession no. U90548), and butyrophilin (Byn_Human; SEQ ID NO: 19; GenBank accession no. U39576);

Figures 3A-3E illustrate the genomic nucleotide sequence for human B7-L polypeptide (SEQ ID NO: 4). The location of the exons (underline) and the deduced amino acid sequences of the exons (SEQ ID NOS: 5-9) are indicated.

5 Detailed Description of the Invention

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein.

10 Definitions

The terms "B7-L gene" or "B7-L nucleic acid molecule" or "B7-L polynucleotide" refer to a nucleic acid molecule comprising or consisting of a nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2, and nucleic acid molecules as defined 15 herein.

The term "B7-L polypeptide allelic variant" refers to one of several possible naturally occurring alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "B7-L polypeptide splice variant" refers to a nucleic acid molecule, 20 usually RNA, which is generated by alternative processing of intron sequences in an RNA transcript of B7-L polypeptide amino acid sequence as set forth in SEQ ID NO: 2.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of 25 the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated 30 nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "B7-L polypeptide" refers to a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 and related polypeptides. Related polypeptides include B7-L polypeptide fragments, B7-L polypeptide orthologs, B7-L polypeptide variants, and B7-L polypeptide derivatives, which possess at least one activity of the polypeptide as set forth in SEQ ID NO: 2. B7-L polypeptides may be mature polypeptides, as defined herein, and may or may not have an amino-terminal methionine residue, depending on the method by which they are prepared.

The term "B7-L polypeptide fragment" refers to a polypeptide that comprises a truncation at the amino-terminus (with or without a leader sequence) and/or a truncation at the carboxyl-terminus of the polypeptide as set forth in SEQ ID NO: 2. The term "B7-L polypeptide fragment" also refers to amino-terminal and/or carboxyl-terminal truncations of B7-L polypeptide orthologs, B7-L polypeptide derivatives, or B7-L polypeptide variants, or to amino-terminal and/or carboxyl-terminal truncations of the polypeptides encoded by B7-L polypeptide allelic variants or B7-L polypeptide splice variants. B7-L polypeptide fragments may result from alternative RNA splicing or from *in vivo* protease activity. Membrane-bound forms of a B7-L polypeptide are also contemplated by the present invention. In preferred embodiments, truncations and/or deletions comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments so produced will comprise about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids, or about 200 amino acids. Such B7-L polypeptide fragments may optionally comprise an amino-terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies to B7-L polypeptides.

The term "B7-L polypeptide ortholog" refers to a polypeptide from another species that corresponds to B7-L polypeptide amino acid sequence as set forth in SEQ ID NO: 2. For example, mouse and human B7-L polypeptides are considered orthologs of each other.

The term "B7-L polypeptide variants" refers to B7-L polypeptides comprising amino acid sequences having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or B7-L polypeptide fragments), and/or additions (such as internal additions and/or B7-L fusion polypeptides) as compared to 5 the B7-L polypeptide amino acid sequence set forth in SEQ ID NO: 2 (with or without a leader sequence). Variants may be naturally occurring (e.g., B7-L polypeptide allelic variants, B7-L polypeptide orthologs, and B7-L polypeptide splice variants) or artificially constructed. Such B7-L polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies 10 accordingly from the DNA sequence as set forth in SEQ ID NO: 1. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, 15 wherein the substitutions may be conservative, or non-conservative, or any combination thereof.

The term "B7-L polypeptide derivatives" refers to the polypeptide as set forth in SEQ ID NO: 2, B7-L polypeptide fragments, B7-L polypeptide orthologs, or B7-L polypeptide variants, as defined herein, that have been chemically modified. The term "B7-L polypeptide derivatives" also refers to the polypeptides encoded by B7-L polypeptide allelic variants or B7-L polypeptide splice variants, as defined herein, 20 that have been chemically modified.

The term "mature B7-L polypeptide" refers to a B7-L polypeptide lacking a leader sequence. A mature B7-L polypeptide may also include other modifications such as proteolytic processing of the amino-terminus (with or without a leader 25 sequence) and/or the carboxyl-terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like. An exemplary mature B7-L polypeptide is depicted by the amino acid sequence of SEQ ID NO: 3.

The term "B7-L fusion polypeptide" refers to a fusion of one or more amino acids (such as a heterologous protein or peptide) at the amino- or carboxyl-terminus 30 of the polypeptide as set forth in SEQ ID NO: 2, B7-L polypeptide fragments, B7-L polypeptide orthologs, B7-L polypeptide variants, or B7-L derivatives, as defined herein. The term "B7-L fusion polypeptide" also refers to a fusion of one or more amino acids at the amino- or carboxyl-terminus of the polypeptide encoded by B7-L polypeptide allelic variants or B7-L polypeptide splice variants, as defined herein.

The term "biologically active B7-L polypeptides" refers to B7-L polypeptides having at least one activity characteristic of the polypeptide comprising the amino acid sequence of SEQ ID NO: 2. In addition, a B7-L polypeptide may be active as an immunogen; that is, the B7-L polypeptide contains at least one epitope to which 5 antibodies may be raised.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or 10 noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any 15 other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "identity," as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also 20 means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer 25 program (*i.e.*, "algorithms").

The term "similarity" is a related concept, but in contrast to "identity," "similarity" refers to a measure of relatedness that includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative 30 substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are five more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the

percent similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a B7-L polypeptide or B7-L nucleic acid molecule used to support an observable level of one or more biological activities of the B7-L polypeptides as set forth herein.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of the B7-L polypeptide, B7-L nucleic acid molecule, or B7-L selective binding agent as a pharmaceutical composition.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "selective binding agent" refers to a molecule or molecules having specificity for a B7-L polypeptide. As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human B7-L polypeptides and not to bind to human non-B7-L polypeptides. It will be appreciated, however, that the selective binding agents may also bind orthologs of the polypeptide as set forth in SEQ ID NO: 2, that is, interspecies versions thereof, such as mouse and rat B7-L polypeptides.

The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham *et al.*, 1973, *Virology*

52:456; Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratories, 1989); Davis *et al.*, *Basic Methods in Molecular Biology* (Elsevier, 1986); and Chu *et al.*, 1981, *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

5 The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the
10 cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

Relatedness of Nucleic Acid Molecules and/or Polypeptides

15 It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of SEQ ID NO: 1, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or deletion of one
20 or more amino acid residues compared to the polypeptide as set forth in SEQ ID NO: 2. Such related B7-L polypeptides may comprise, for example, an addition and/or a deletion of one or more N-linked or O-linked glycosylation sites or an addition and/or a deletion of one or more cysteine residues.

25 Related nucleic acid molecules also include fragments of B7-L nucleic acid molecules which encode a polypeptide of at least about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids, or about 200 amino acids, or more than 200 amino acid residues of the B7-L polypeptide of SEQ ID NO: 2.

30 In addition, related B7-L nucleic acid molecules also include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the B7-L nucleic acid molecule of SEQ ID NO: 1, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 2, or of a nucleic acid fragment as defined herein, or of a nucleic acid

fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the B7-L sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of B7-L polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein and those regions may be used to design probes for screening.

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory, 1989); Anderson *et al.*, *Nucleic Acid Hybridisation: A Practical Approach* Ch. 4 (IRL Press Limited).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used— however, the rate of hybridization will be affected. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDODSO₄, (SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or another non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4; however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson *et al.*, *Nucleic Acid Hybridisation: A Practical Approach* Ch. 4 (IRL Press Limited).

Factors affecting the stability of DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of

different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$T_m(\text{°C}) = 81.5 + 16.6(\log[\text{Na}^+]) + 0.41(\%G+C) - 600/N - 0.72(\%\text{formamide})$$

where N is the length of the duplex formed, $[\text{Na}^+]$ is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under 10 "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015 M sodium chloride, 0.0015 M sodium citrate at 50-65°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 20% formamide at 37-50°C. By way of example, "moderately stringent conditions" of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

15 It will be appreciated by those skilled in the art that there is no absolute distinction between "highly stringent conditions" and "moderately stringent conditions." For example, at 0.015 M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To 20 capture more distantly related sequences, one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl* for oligonucleotide probes up to about 20nt is given by:

$$T_m = 2^\circ\text{C per A-T base pair} + 4^\circ\text{C per G-C base pair}$$

25 *The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs *et al., Developmental Biology Using Purified Genes* 683 (Brown and Fox, eds., 1981).

High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5°C below the T_m of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of 30 a nucleotide sequence that is at least about 70 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or comprise or consist essentially of a nucleotide sequence encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in SEQ ID NO: 2. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent,

or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequence as set forth in SEQ ID NO: 2. Related nucleic acid molecules encode polypeptides possessing at least one activity of the polypeptide set forth in SEQ ID NO: 2.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of the amino acid sequence relative to the amino acid sequence of SEQ ID NO: 2.

Conservative modifications to the amino acid sequence of SEQ ID NO: 2 (and the corresponding modifications to the encoding nucleotides) will produce a polypeptide having functional and chemical characteristics similar to those of B7-L polypeptides. In contrast, substantial modifications in the functional and/or chemical characteristics of B7-L polypeptides may be accomplished by selecting substitutions in the amino acid sequence of SEQ ID NO: 2 that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues may be divided into classes based on common side chain properties:

- 1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- 2) neutral hydrophilic: Cys, Ser, Thr;
- 3) acidic: Asp, Glu;
- 4) basic: Asn, Gln, His, Lys, Arg;

- 5) residues that influence chain orientation: Gly, Pro; and
- 6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted 5 residues may be introduced into regions of the human B7-L polypeptide that are homologous with non-human B7-L polypeptides, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of 10 its hydrophobicity and charge characteristics. The hydropathic indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-15 4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte *et al.*, 1982, *J. Mol. Biol.* 157:105-31). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar 20 biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be 25 made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological 30 property of the protein.

The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3);

valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (2.5); and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even 5 more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. 10 For example, amino acid substitutions can be used to identify important residues of the B7-L polypeptide, or to increase or decrease the affinity of the B7-L polypeptides described herein. Exemplary amino acid substitutions are set forth in Table I.

Table I
Amino Acid Substitutions

15

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu

Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in SEQ ID NO: 2 using well-known techniques. For identifying suitable areas of the molecule that may be changed without destroying biological activity, one 5 skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a B7-L polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are 10 conserved among similar polypeptides. It will be appreciated that changes in areas of the B7-L molecule that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of a B7-L polypeptide. One skilled in the art would also know that, even in relatively conserved 15 regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies 20 identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a B7-L polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for

chemically similar amino acid substitutions for such predicted important amino acid residues of B7-L polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of B7-L polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each amino acid residue. The variants could be screened using activity assays known to those with skill in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult, 1996, *Curr. Opin. Biotechnol.* 7:422-27; Chou *et al.*, 1974, *Biochemistry* 13:222-45; Chou *et al.*, 1974, *Biochemistry* 13:211-22; Chou *et al.*, 1978, *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45-48; Chou *et al.*, 1978, *Ann. Rev. Biochem.* 47:251-276; and Chou *et al.*, 1979, *Biophys. J.* 26:367-84. Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins that have a sequence identity of greater than 30%, or similarity greater than 40%, often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within the structure of a polypeptide or protein. See Holm *et al.*, 1999, *Nucleic Acids Res.* 27:244-47. It has been suggested that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate (Brenner *et al.*, 1997, *Curr. Opin. Struct. Biol.* 7:369-76).

Additional methods of predicting secondary structure include "threading" (Jones, 1997, *Curr. Opin. Struct. Biol.* 7:377-87; Sippl *et al.*, 1996, *Structure* 4:15-19), "profile analysis" (Bowie *et al.*, 1991, *Science*, 253:164-70; Gribskov *et al.*, 1990, *Methods Enzymol.* 183:146-59; Gribskov *et al.*, 1987, *Proc. Nat. Acad. Sci. U.S.A.* 84:4355-58), and "evolutionary linkage" (See Holm *et al.*, *supra*, and Brenner *et al.*, *supra*).

Preferred B7-L polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites have been altered compared to the amino acid sequence set forth in SEQ ID NO: 2. In one embodiment, B7-L polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than the amino acid sequence set forth in SEQ ID NO: 2. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions that eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created.

Additional preferred B7-L variants include cysteine variants, wherein one or more cysteine residues are deleted or substituted with another amino acid (e.g., serine) as compared to the amino acid sequence set forth in SEQ ID NO: 2. Cysteine variants are useful when B7-L polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In other embodiments, related nucleic acid molecules comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid insertion and wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2, or a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid deletion and wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2. Related nucleic acid molecules also comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 wherein the polypeptide has a

carboxyl- and/or amino-terminal truncation and further wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2. Related nucleic acid molecules also comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one modification selected from the group 5 consisting of amino acid substitutions, amino acid insertions, amino acid deletions, carboxyl-terminal truncations, and amino-terminal truncations and wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2.

In addition, the polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or other B7-L polypeptide, may be fused to a homologous polypeptide to form 10 a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of a B7-L fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane 15 receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide comprising the amino acid sequence as set forth in 20 SEQ ID NO: 2, or other B7-L polypeptide.

Fusions can be made either at the amino-terminus or at the carboxyl-terminus 25 of the polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2, or other B7-L polypeptide. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically from about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

30 In a further embodiment of the invention, the polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or other B7-L polypeptide, is fused to one or more domains of an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab," that binds an antigen, and a constant domain known as "Fc," that is involved in effector functions such as

complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon *et al.*, 1989, *Nature* 337:525-31. When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding, complement fixation, and perhaps even placental transfer. *Id.* Table II summarizes the use of certain Fc fusions known in the art.

Table II
Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fc γ 2a	IL-10	anti-inflammatory; transplant rejection	Zheng <i>et al.</i> , 1995, <i>J. Immunol.</i> 154:5590-600
IgG1	TNF receptor	septic shock	Fisher <i>et al.</i> , 1996, <i>N. Engl. J. Med.</i> 334:1697-1702; Van Zee <i>et al.</i> , 1996, <i>J. Immunol.</i> 156:2221-30
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Patent No. 5,808,029
IgG1	CD4 receptor	AIDS	Capon <i>et al.</i> , 1989, <i>Nature</i> 337: 525-31
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill <i>et al.</i> , 1995, <i>Immunotech.</i> 1:95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cy1	CTLA-4	autoimmune disorders	Linsley, 1991, <i>J. Exp. Med.</i> , 174:561-69

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In one example, a human IgG hinge, CH2, and CH3 region may be fused at either the amino-terminus or carboxyl-terminus of the B7-L polypeptides using methods known to the skilled artisan. In another example, a human IgG hinge, CH2, and CH3 region may be fused at either the amino-terminus or carboxyl-terminus of a 15 B7-L polypeptide fragment (e.g., the predicted extracellular portion of B7-L polypeptide).

The resulting B7-L fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion 5 polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, or reduced aggregation.

Identity and similarity of related nucleic acid molecules and polypeptides are readily calculated by known methods. Such methods include, but are not limited to 10 those described in *Computational Molecular Biology* (A.M. Lesk, ed., Oxford University Press 1988); *Biocomputing: Informatics and Genome Projects* (D.W. Smith, ed., Academic Press 1993); *Computer Analysis of Sequence Data* (Part 1, A.M. Griffin and H.G. Griffin, eds., Humana Press 1994); G. von Heinle, *Sequence Analysis in Molecular Biology* (Academic Press 1987); *Sequence Analysis Primer* 15 (M. Gribskov and J. Devereux, eds., M. Stockton Press 1991); and Carillo *et al.*, 1988, *SIAM J. Applied Math.*, 48:1073.

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and 20 similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, 1984, *Nucleic Acids Res.* 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10). The BLASTX program is publicly available from the 25 National Center for Biotechnology Information (NCBI) and other sources (Altschul *et al.*, *BLAST Manual* (NCB NLM NIH, Bethesda, MD); Altschul *et al.*, 1990, *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result 30 in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the claimed polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span," as determined by the algorithm). A gap 5 opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 0.1X the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 10 62 are used in conjunction with the algorithm. A standard comparison matrix is also used by the algorithm (see Dayhoff *et al.*, 5 *Atlas of Protein Sequence and Structure* (Supp. 3 1978)(PAM250 comparison matrix); Henikoff *et al.*, 1992, *Proc. Natl. Acad. Sci USA* 89:10915-19 (BLOSUM 62 comparison matrix)).

Preferred parameters for polypeptide sequence comparison include the 15 following:

Algorithm: Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443-53;
Comparison matrix: BLOSUM 62 (Henikoff *et al., supra*);
Gap Penalty: 12
20 Gap Length Penalty: 4
Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned 25 parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparison include the following:

Algorithm: Needleman and Wunsch, *supra*;
30 Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
Gap Length Penalty: 3

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, and thresholds of similarity may be used, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA-to-DNA, protein-to-protein, protein-to-DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Nucleic Acid Molecules

The nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of a B7-L polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening, and/or PCR amplification of cDNA.

Recombinant DNA methods used herein are generally those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989) and/or *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1994). The invention provides for nucleic acid molecules as described herein and methods for obtaining such molecules.

Where a gene encoding the amino acid sequence of a B7-L polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify orthologs or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the B7-L polypeptide. In addition, part or all of a nucleic acid molecule having the sequence as set forth in SEQ ID NO: 1 may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence of a B7-L polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screening.

Nucleic acid molecules encoding the amino acid sequence of B7-L polypeptides may also be identified by expression cloning which employs the

detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding an antibody or other binding partner (e.g., receptor or ligand) to cloned proteins that are expressed and displayed on a host cell surface. The antibody or binding partner is modified with a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence that encodes the amino acid sequence of a B7-L polypeptide into an appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of a B7-L polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate host, the encoded B7-L polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA encoding the amino acid sequence of a B7-L polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of a B7-L polypeptide is chemical synthesis using methods well known to the skilled artisan such as those described by Engels *et al.*, 1989, *Angew. Chem. Int'l. Ed.* 28:716-34. These methods include, *inter alia*, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of a B7-L polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full-length nucleotide sequence of a B7-L gene. Usually, the DNA fragment encoding the amino-terminus of the polypeptide will have an ATG, which encodes a methionine

residue. This methionine may or may not be present on the mature form of the B7-L polypeptide, depending on whether the polypeptide produced in the host cell is designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

5 In certain embodiments, nucleic acid variants contain codons which have been altered for optimal expression of a B7-L polypeptide in a given host cell. Particular codon alterations will depend upon the B7-L polypeptide and host cell selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed 10 genes in a given host cell. Computer algorithms which incorporate codon frequency tables such as "Eco_high.Cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0 (Genetics Computer Group, Madison, WI). Other useful codon frequency tables include "Celegans_high.cod," "Celegans_low.cod," "Drosophila_high.cod," 15 "Human_high.cod," "Maize_high.cod," and "Yeast_high.cod."

In some cases, it may be desirable to prepare nucleic acid molecules encoding B7-L polypeptide variants. Nucleic acid molecules encoding variants may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point mutations (*see* Sambrook *et al.*, 20 *supra*, and Ausubel *et al.*, *supra*, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels *et al.*, *supra*, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

25 **Vectors and Host Cells**

A nucleic acid molecule encoding the amino acid sequence of a B7-L polypeptide is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that 30 amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding the amino acid sequence of a B7-L polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether a B7-L polypeptide is to be post-translationally modified (*e.g.*, glycosylated and/or

phosphorylated). If so, yeast, insect, or mammalian host cells are preferable. For a review of expression vectors, see *Meth. Enz.*, vol. 185 (D.V. Goeddel, ed., Academic Press 1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, i.e., an oligonucleotide molecule located at the 5' or 3' end of the B7-L polypeptide coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or another "tag" such as FLAG, HA (hemagglutinin influenza virus), or myc for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the B7-L polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified B7-L polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more than one source), or synthetic, or the flanking sequences may be native sequences that normally function to regulate B7-L polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

Flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein – other than the B7-L gene flanking sequences – will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with a suitable oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen[®] column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of a B7-L polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Beverly, MA) is suitable for most gram-negative bacteria and various origins (e.g., SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment

followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the survival
5 and growth of a host cell grown in a selective culture medium. Typical selection
marker genes encode proteins that (a) confer resistance to antibiotics or other toxins,
e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement
auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from
complex media. Preferred selectable markers are the kanamycin resistance gene, the
10 ampicillin resistance gene, and the tetracycline resistance gene. A neomycin
resistance gene may also be used for selection in prokaryotic and eukaryotic host
cells.

Other selection genes may be used to amplify the gene that will be expressed.
Amplification is the process wherein genes that are in greater demand for the
15 production of a protein critical for growth are reiterated in tandem within the
chromosomes of successive generations of recombinant cells. Examples of suitable
selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and
thymidine kinase. The mammalian cell transformants are placed under selection
pressure wherein only the transformants are uniquely adapted to survive by virtue of
20 the selection gene present in the vector. Selection pressure is imposed by culturing
the transformed cells under conditions in which the concentration of selection agent in
the medium is successively changed, thereby leading to the amplification of both the
selection gene and the DNA that encodes a B7-L polypeptide. As a result, increased
quantities of B7-L polypeptide are synthesized from the amplified DNA.

25 A ribosome binding site is usually necessary for translation initiation of
mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak
sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to
the coding sequence of a B7-L polypeptide to be expressed. The Shine-Dalgarno
sequence is varied but is typically a polypurine (i.e., having a high A-G content).
30 Many Shine-Dalgarno sequences have been identified, each of which can be readily
synthesized using methods set forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct a B7-L polypeptide out of
the host cell. Typically, a nucleotide sequence encoding the signal sequence is
positioned in the coding region of a B7-L nucleic acid molecule, or directly at the 5'

end of a B7-L polypeptide coding region. Many signal sequences have been identified, and any of those that are functional in the selected host cell may be used in conjunction with a B7-L nucleic acid molecule. Therefore, a signal sequence may be homologous (naturally occurring) or heterologous to the B7-L nucleic acid molecule.

5 Additionally, a signal sequence may be chemically synthesized using methods described herein. In most cases, the secretion of a B7-L polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted B7-L polypeptide. The signal sequence may be a component of the vector, or it may be a part of a B7-L nucleic acid molecule that is inserted into the

10 vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native B7-L polypeptide signal sequence joined to a B7-L polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to a B7-L polypeptide coding region. The heterologous signal sequence selected should be one that is recognized and processed, *i.e.*, cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native B7-L polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native B7-L polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

25 In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add pro-sequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression,

30 which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired B7-L polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the B7-L gene especially where the gene used is a full-length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron may be obtained from another source. The position of the intron with respect to flanking sequences and the B7-L gene is generally important, as the intron must be transcribed to be effective. Thus, when a B7-L cDNA molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site and 5' to the polyA transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (*i.e.*, 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding the B7-L polypeptide. Promoters are untranscribed sequences located upstream (*i.e.*, 5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding B7-L polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native B7-L promoter sequence may be used to direct amplification and/or expression of a B7-L nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of

the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase; a tryptophan (*trp*) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence, using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art.

10 Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling B7-L gene expression include, but are not limited to: the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* 290:304-10); the CMV promoter; the promoter contained 20 in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-97); the herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1444-45); the regulatory sequences of the metallothioneine gene (Brinster *et al.*, 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.*, 25 75:3727-31); or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-46; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 30 50:399-409 (1986); MacDonald, 1987, *Hepatology* 7:425-515); the insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-22); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-58; Adames *et al.*, 1985, *Nature* 318:533-38; Alexander *et al.*, 1987, *Mol. Cell. Biol.*, 7:1436-44); the mouse mammary tumor

virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, *Cell* 45:485-95); the albumin gene control region which is active in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1:268-76); the alpha-feto-protein gene control region which is active in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.*, 5:1639-48; Hammer *et al.*, 1987, *Science* 235:53-58); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, *Genes and Devel.* 1:161-71); the beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, 1985, *Nature* 315:338-40; Kollias *et al.*, 1986, *Cell* 46:89-94); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, *Cell* 48:703-12); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-86); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, *Science* 234:1372-78).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding a B7-L polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to a B7-L nucleic acid molecule, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Preferred vectors for practicing this invention are those that are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen, San Diego, CA), pBSII (Stratagene, La

Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII, Invitrogen), pDSR-alpha (PCT Pub. No. WO 90/14363) and pFastBacDual (Gibco-BRL, Grand Island, NY).

5 Additional suitable vectors include, but are not limited to, cosmids, plasmids, or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript[®] plasmid derivatives (a high copy number ColE1-based phagemid; Stratagene Cloning Systems, La Jolla CA), PCR cloning plasmids 10 designed for cloning Taq-amplified PCR products (e.g., TOPO[™] TA Cloning[®] Kit and PCR2.1[®] plasmid derivatives; Invitrogen), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives; Clontech).

15 After the vector has been constructed and a nucleic acid molecule encoding a B7-L polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for a B7-L polypeptide into a selected host cell may be accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection, lipofection, DEAE-dextran method, or other known techniques. The method selected 20 will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

25 Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast, insect, or vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes a B7-L polypeptide that can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity (such as 30 glycosylation or phosphorylation) and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), Manassas, VA. Examples

include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO), CHO DHFR(-) cells (Urlaub *et al.*, 1980, *Proc. Natl. Acad. Sci. U.S.A.* 77:4216-20), human embryonic kidney (HEK) 293 or 293T cells, or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, 5 amplification, screening, product production, and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are 10 also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell 15 lines. Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, DH5 α , DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like 20 may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerevisiae* and *Pichia pastoris*.

Additionally, where desired, insect cell systems may be utilized in the 25 methods of the present invention. Such systems are described, for example, in Kitts *et al.*, 1993, *Biotechniques*, 14:810-17; Lucklow, 1993, *Curr. Opin. Biotechnol.* 4:564-72; and Lucklow *et al.*, 1993, *J. Virol.*, 67:4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen).

One may also use transgenic animals to express glycosylated B7-L 30 polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce B7-L polypeptides, however, in general, the glycosylation occurring in plants is different from that produced in mammalian

cells, and may result in a glycosylated product which is not suitable for human therapeutic use.

Polypeptide Production

5 Host cells comprising a B7-L polypeptide expression vector may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells include, for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells include Roswell
10 Park Memorial Institute medium 1640 (RPMI 1640), Minimal Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium (DMEM), all of which may be supplemented with serum and/or growth factors as necessary for the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as
15 necessary.

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable
20 marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline, and neomycin.

The amount of a B7-L polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation,
25 Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, High Performance Liquid Chromatography (HPLC) separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If a B7-L polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. If however, the
30 B7-L polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for gram-negative bacteria host cells).

For a B7-L polypeptide situated in the host cell cytoplasm and/or nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), the intracellular

material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by 5 centrifugation.

If a B7-L polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with a chaotropic agent such as a detergent, 10 guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The solubilized B7-L polypeptide can then be analyzed using gel electrophoresis, immunoprecipitation, or the like. If it is desired to isolate the B7-L polypeptide, 15 isolation may be accomplished using standard methods such as those described herein and in Marston *et al.*, 1990, *Meth. Enz.*, 182:264-75.

In some cases, a B7-L polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological 20 activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the 25 refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridges. Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, 30 and 2-2-mercaptoethanol(bME)/dithio-b(MB). In many instances, a cosolvent may be used or may be needed to increase the efficiency of the refolding, and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of a B7-L polypeptide, then the polypeptide will be found primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described herein.

5 The purification of a B7-L polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (B7-L polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen) at either its carboxyl- or amino-terminus, it may be purified in a one-step process by
10 passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel. Thus, an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of B7-L polypeptide/polyHis. See, e.g., *Current Protocols in Molecular Biology* § 10.11.8 (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1993).

Additionally, B7-L polypeptides may be purified through the use of a monoclonal antibody that is capable of specifically recognizing and binding to a B7-L polypeptide.

20 Other suitable procedures for purification include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, HPLC, electrophoresis (including native gel electrophoresis) followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

B7-L polypeptides may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield *et al.*, 1963, *J. Am. Chem. Soc.* 85:2149; Houghten *et al.*, 1985,
30 *Proc Natl Acad. Sci. USA* 82:5132; and Stewart and Young, *Solid Phase Peptide Synthesis* (Pierce Chemical Co. 1984). Such polypeptides may be synthesized with or without a methionine on the amino-terminus. Chemically synthesized B7-L polypeptides may be oxidized using methods set forth in these references to form disulfide bridges. Chemically synthesized B7-L polypeptides are expected to have

comparable biological activity to the corresponding B7-L polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with a recombinant or natural B7-L polypeptide.

Another means of obtaining B7-L polypeptide is via purification from 5 biological samples such as source tissues and/or fluids in which the B7-L polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described herein. The presence of the B7-L polypeptide during purification may be monitored, for example, using an antibody prepared against recombinantly produced B7-L polypeptide or peptide fragments thereof.

10 A number of additional methods for producing nucleic acids and polypeptides are known in the art, and the methods can be used to produce polypeptides having specificity for B7-L polypeptide. *See, e.g.,* Roberts *et al.*, 1997, *Proc. Natl. Acad. Sci. U.S.A.* 94:12297-303, which describes the production of fusion proteins between an mRNA and its encoded peptide. *See also,* Roberts, 1999, *Curr. Opin. Chem. Biol.* 3:268-73. Additionally, U.S. Patent No. 5,824,469 describes methods for obtaining 15 oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous pool of oligonucleotides, each having a 5' randomized sequence, a central preselected sequence, and a 3' randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not 20 exhibit the desired biological function. Subpopulations of the cells are then screened for those that exhibit a predetermined biological function. From that subpopulation, oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,763,192; 5,814,476; 5,723,323; and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing 25 stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Another method for producing peptides or polypeptides is described in 30 PCT/US98/20094 (WO99/15650) filed by Athersys, Inc. Known as "Random Activation of Gene Expression for Gene Discovery" (RAGE-GD), the process involves the activation of endogenous gene expression or over-expression of a gene by *in situ* recombination methods. For example, expression of an endogenous gene is activated or increased by integrating a regulatory sequence into the target cell that is

capable of activating expression of the gene by non-homologous or illegitimate recombination. The target DNA is first subjected to radiation, and a genetic promoter inserted. The promoter eventually locates a break at the front of a gene, initiating transcription of the gene. This results in expression of the desired peptide or 5 polypeptide.

It will be appreciated that these methods can also be used to create comprehensive B7-L polypeptide expression libraries, which can subsequently be used for high throughput phenotypic screening in a variety of assays, such as biochemical assays, cellular assays, and whole organism assays (e.g., plant, mouse, 10 etc.).

Synthesis

It will be appreciated by those skilled in the art that the nucleic acid and 15 polypeptide molecules described herein may be produced by recombinant and other means.

Selective Binding Agents

The term "selective binding agent" refers to a molecule that has specificity for one or more B7-L polypeptides. Suitable selective binding agents include, but are not 20 limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable selective binding agents may be prepared using methods known in the art. An exemplary B7-L polypeptide selective binding agent of the present invention is capable of binding a certain portion of the B7-L polypeptide thereby inhibiting the binding of the polypeptide to a B7-L polypeptide receptor.

25 Selective binding agents such as antibodies and antibody fragments that bind B7-L polypeptides are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal; monoclonal (MAbs); recombinant; chimeric; humanized, such as complementarity-determining region (CDR)-grafted; human; single chain; and/or bispecific; as well as fragments; variants; or derivatives 30 thereof. Antibody fragments include those portions of the antibody that bind to an epitope on the B7-L polypeptide. Examples of such fragments include Fab and F(ab')² fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding

antibody variable regions.

5 Polyclonal antibodies directed toward a B7-L polypeptide generally are produced in animals (e.g., rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of B7-L polypeptide and an adjuvant. It may be useful to conjugate a B7-L polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-B7-L antibody titer.

10 Monoclonal antibodies directed toward B7-L polypeptides are produced using any method that provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, 1975, *Nature* 256:495-97 and the human B-cell hybridoma method (Kozbor, 1984, *J. Immunol.* 133:3001; Brodeur *et* 15 *al.*, *Monoclonal Antibody Production Techniques and Applications* 51-63 (Marcel Dekker, Inc., 1987). Also provided by the invention are hybridoma cell lines that produce monoclonal antibodies reactive with B7-L polypeptides.

20 Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy (H) and/or light (L) chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of 25 such antibodies, so long as they exhibit the desired biological activity. See U.S. Patent No. 4,816,567; Morrison *et al.*, 1985, *Proc. Natl. Acad. Sci.* 81:6851-55.

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089 and 5,693,762. Generally, a 30 humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. Humanization can be performed, for example, using methods described in the art (Jones *et al.*, 1986, *Nature* 321:522-25; Riechmann *et al.*, 1998, *Nature* 332:323-27; Verhoeyen *et al.*, 1988, *Science* 239:1534-36), by

substituting at least a portion of a rodent complementarity-determining region for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies that bind B7-L polypeptides. Using transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies are produced by immunization with a B7-L polypeptide antigen (i.e., having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, e.g., Jakobovits *et al.*, 1993, *Proc. Natl. Acad. Sci.* 90:2551-55; Jakobovits *et al.*, 1993, *Nature* 362:255-58; Brugermann *et al.*, 1993, *Year in Immunol.* 7:33. In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals (i.e., those having less than the full complement of modifications) are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than, e.g., murine) amino acid sequences, including variable regions that are immunospecific for these antigens. See PCT App. Nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT App. Nos. PCT/US91/245 and PCT/GB89/01207, and in European Patent Nos. 546073B1 and 546073A1. Human antibodies can also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can also be produced from phage-display libraries (Hoogenboom *et al.*, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT App. No. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic antibodies for MPL and msk-receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO

cells. Monoclonal (e.g., human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-B7-L antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (*Sola, Monoclonal Antibodies: A Manual of Techniques* 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of B7-L polypeptides. The antibodies will bind B7-L polypeptides with an affinity that is appropriate for the assay method being employed.

For diagnostic applications, in certain embodiments, anti-B7-L antibodies may be labeled with a detectable moiety. The detectable moiety can be any one that is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , ^{125}I , ^{99}Tc , ^{111}In , or ^{67}Ga ; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β -galactosidase, or horseradish peroxidase (Bayer, *et al.*, 1990, *Meth. Enz.* 184:138-63).

Competitive binding assays rely on the ability of a labeled standard (e.g., a B7-L polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (an B7-L polypeptide) for binding with a limited amount of anti-B7-L antibody. The amount of a B7-L polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte that remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody that is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich

assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-B7-L antibodies, are also useful for *in vivo* imaging. An antibody labeled with a detectable moiety may be 5 administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host assayed. The antibody may be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

Selective binding agents of the invention, including antibodies, may be used 10 as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of a B7-L polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to a B7-L polypeptide and which are capable of inhibiting or eliminating the functional 15 activity of a B7-L polypeptide *in vivo* or *in vitro*. In preferred embodiments, the selective binding agent, e.g., an antagonist antibody, will inhibit the functional activity of a B7-L polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an anti-B7-L polypeptide antibody that is capable of interacting with a B7-L polypeptide binding 20 partner (a ligand or receptor) thereby inhibiting or eliminating B7-L polypeptide activity *in vitro* or *in vivo*. Selective binding agents, including agonist and antagonist anti-B7-L polypeptide antibodies, are identified by screening assays that are well known in the art.

The invention also relates to a kit comprising B7-L selective binding agents 25 (such as antibodies) and other reagents useful for detecting B7-L polypeptide levels in biological samples. Such reagents may include a detectable label, blocking serum, positive and negative control samples, and detection reagents.

Microarrays

30 It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high-density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array contains numerous copies of a single nucleic acid species that acts as a target for hybridization with a complementary nucleic acid sequence.

(e.g., mRNA). In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented 5 on the array is then visualized by quantitating the amount of labeled cDNA that is specifically bound to each target nucleic acid molecule. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications 10 with respect to the B7-L molecules of the invention, including, but not limited to: the identification and validation of B7-L disease-related genes as targets for therapeutics; molecular toxicology of related B7-L molecules and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and enhancing related B7-L polypeptide small molecule drug discovery by aiding in the 15 identification of selective compounds in high throughput screens.

Chemical Derivatives

Chemically modified derivatives of B7-L polypeptides may be prepared by one skilled in the art, given the disclosures described herein. B7-L polypeptide 20 derivatives are modified in a manner that is different—either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. The polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or other B7-L polypeptide, may be modified by the covalent attachment of one or more polymers. 25 For example, the polymer selected is typically water-soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

30 The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an average molecular weight of between about 2 kDa to about 100 kDa (the term “about” indicating that in preparations of a water-soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer is

preferably between about 5 kDa and about 50 kDa, more preferably between about 12 kDa and about 40 kDa and most preferably between about 20 kDa and about 35 kDa.

Suitable water-soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C₁-C₁₀), alkoxy-, or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran of, for example, about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxycetylated polyols (e.g., glycerol), and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules that may be used to prepare covalently attached B7-L polypeptide multimers.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides will generally comprise the steps of: (a) reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby the polypeptide comprising the amino acid sequence of SBQ ID NO: 2, or other B7-L polypeptide, becomes attached to one or more polymer molecules, and (b) obtaining the reaction products. The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer molecules to protein, the greater the percentage of attached polymer molecule. In one embodiment, the B7-L polypeptide derivative may have a single polymer molecule moiety at the amino-terminus. See, e.g., U.S. Patent No. 5,234,784.

The pegylation of a polypeptide may be specifically carried out using any of the pegylation reactions known in the art. Such reactions are described, for example, in the following references: Francis *et al.*, 1992, *Focus on Growth Factors* 3:4-10; European Patent Nos. 0154316 and 0401384; and U.S. Patent No. 4,179,337. For example, pegylation may be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, a selected polymer should have a single reactive ester group. For reductive alkylation, a selected polymer should have a single reactive aldehyde group. A reactive aldehyde

is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C₁-C₁₀ alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

In another embodiment, B7-L polypeptides may be chemically coupled to biotin. The biotin/B7-L polypeptide molecules are then allowed to bind to avidin, 5 resulting in tetravalent avidin/biotin/B7-L polypeptide molecules. B7-L polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions that may be alleviated or modulated by the 10 administration of the present B7-L polypeptide derivatives include those described herein for B7-L polypeptides. However, the B7-L polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

15

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents; rabbits, goats, sheep, or other farm animals, in which the genes encoding native B7-L polypeptide have been 20 disrupted (i.e., "knocked out") such that the level of expression of B7-L polypeptide is significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals such as mice, rats, or other rodents; rabbits, goats, sheep, or other farm animals, in which either the 25 native form of a B7-L gene for that animal or a heterologous B7-L gene is over-expressed by the animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT Pub. No. WO 94/28122.

The present invention further includes non-human animals in which the 30 promoter for one or more of the B7-L polypeptides of the present invention is either activated or inactivated (e.g., by using homologous recombination methods) to alter the level of expression of one or more of the native B7-L polypeptides.

These non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured. For

example, drug candidates may decrease or increase the expression of the B7-L gene. In certain embodiments, the amount of B7-L polypeptide that is produced may be measured after the exposure of the animal to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the 5 animal. For example, over-expression of a particular gene may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene or its ability to prevent or inhibit a pathological condition. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated 10 with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product or its ability to prevent or inhibit a pathological condition.

Assaying for Other Modulators of B7-L Polypeptide Activity

15 In some situations, it may be desirable to identify molecules that are modulators, *i.e.*, agonists or antagonists, of the activity of B7-L polypeptide. Natural or synthetic molecules that modulate B7-L polypeptide may be identified using one or more screening assays, such as those described herein. Such molecules may be administered either in an *ex vivo* manner or in an *in vivo* manner by injection, or by 20 oral delivery, implantation device, or the like.

“Test molecule” refers to a molecule that is under evaluation for the ability to modulate (*i.e.*, increase or decrease) the activity of a B7-L polypeptide. Most commonly, a test molecule will interact directly with a B7-L polypeptide. However, it is also contemplated that a test molecule may also modulate B7-L polypeptide 25 activity indirectly, such as by affecting B7-L gene expression, or by binding to a B7-L polypeptide binding partner (*e.g.*, receptor or ligand). In one embodiment, a test molecule will bind to a B7-L polypeptide with an affinity constant of at least about 10^{-6} M, preferably about 10^{-8} M, more preferably about 10^{-9} M, and even more preferably about 10^{-10} M.

30 Methods for identifying compounds that interact with B7-L polypeptides are encompassed by the present invention. In certain embodiments, a B7-L polypeptide is incubated with a test molecule under conditions that permit the interaction of the test molecule with a B7-L polypeptide, and the extent of the interaction is measured.

The test molecule can be screened in a substantially purified form or in a crude mixture.

In certain embodiments, a B7-L polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule that 5 interacts with B7-L polypeptide to regulate its activity. Molecules which regulate B7-L polypeptide expression include nucleic acids which are complementary to nucleic acids encoding a B7-L polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of B7-L polypeptide, and which act as anti-sense regulators of expression.

10 Once a test molecule has been identified as interacting with a B7-L polypeptide, the molecule may be further evaluated for its ability to increase or decrease B7-L polypeptide activity. The measurement of the interaction of a test molecule with B7-L polypeptide may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays, and 15 immunoassays. In general, a test molecule is incubated with a B7-L polypeptide for a specified period of time, and B7-L polypeptide activity is determined by one or more assays for measuring biological activity.

20 The interaction of test molecules with B7-L polypeptides may also be assayed directly using polyclonal or monoclonal antibodies in an immunoassay. Alternatively, modified forms of B7-L polypeptides containing epitope tags as described herein may be used in solution and immunoassays.

25 In the event that B7-L polypeptides display biological activity through an interaction with a binding partner (e.g., a receptor or a ligand), a variety of *in vitro* assays may be used to measure the binding of a B7-L polypeptide to the corresponding binding partner (such as a selective binding agent, receptor, or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of a B7-L polypeptide to its binding partner. In one assay, a B7-L polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled B7-L polypeptide binding partner (for example, iodinated B7-L 30 polypeptide binding partner) and a test molecule can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted for radioactivity, using a scintillation counter, to determine the extent to which the binding partner bound to the B7-L polypeptide. Typically, a molecule will be tested over a range of concentrations, and a series of control wells

lacking one or more elements of the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, i.e., immobilizing B7-L polypeptide binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled B7-L 5 polypeptide, and determining the extent of B7-L polypeptide binding. See, e.g., *Current Protocols in Molecular Biology*, chap. 18 (Ausubel et al., eds., Green Publishers Inc. and Wiley and Sons 1995).

As an alternative to radiolabeling, a B7-L polypeptide or its binding partner may be conjugated to biotin, and the presence of biotinylated protein can then be 10 detected using streptavidin linked to an enzyme, such as horse radish peroxidase (HRP) or alkaline phosphatase (AP), which can be detected colorimetrically, or by fluorescent tagging of streptavidin. An antibody directed to a B7-L polypeptide or to a B7-L polypeptide binding partner, and which is conjugated to biotin, may also be used for purposes of detection following incubation of the complex with enzyme-linked streptavidin linked to AP or HRP. 15

A B7-L polypeptide or a B7-L polypeptide binding partner can also be immobilized by attachment to agarose beads, acrylic beads, or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After 20 incubation, the beads can be precipitated by centrifugation, and the amount of binding between a B7-L polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column with the test molecule and complementary protein passing through the column. The formation of a complex between a B7-L polypeptide and its 25 binding partner can then be assessed using any of the techniques described herein (e.g., radiolabelling or antibody binding).

Another *in vitro* assay that is useful for identifying a test molecule that increases or decreases the formation of a complex between a B7-L polypeptide binding protein and a B7-L polypeptide binding partner is a surface plasmon resonance detector system such as the BIACore assay system (Pharmacia, Piscataway, 30 NJ). The BIACore system is utilized as specified by the manufacturer. This assay essentially involves the covalent binding of either B7-L polypeptide or a B7-L polypeptide binding partner to a dextran-coated sensor chip that is located in a detector. The test compound and the other complementary protein can then be

injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass that is physically associated with the dextran-coated side of the sensor chip, with the change in molecular mass being measured by the detector system.

5 In some cases, it may be desirable to evaluate two or more test compounds together for their ability to increase or decrease the formation of a complex between a B7-L polypeptide and a B7-L polypeptide binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) 10 either simultaneously with, or subsequent to, the first test compound. The remainder of the steps in the assay are as set forth herein.

15 *In vitro* assays such as those described herein may be used advantageously to screen large numbers of compounds for an effect on the formation of a complex between a B7-L polypeptide and B7-L polypeptide binding partner. The assays may be automated to screen compounds generated in phage display, synthetic peptide, and chemical synthesis libraries.

20 Compounds which increase or decrease the formation of a complex between a B7-L polypeptide and a B7-L polypeptide binding partner may also be screened in cell culture using cells and cell lines expressing either B7-L polypeptide or B7-L polypeptide binding partner. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The 25 binding of a B7-L polypeptide to cells expressing B7-L polypeptide binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to a B7-L polypeptide binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For 30 example, drug candidates may decrease or increase the expression of the B7-L gene. In certain embodiments, the amount of B7-L polypeptide or a B7-L polypeptide fragment that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the over-expression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug

candidate's ability to increase or decrease the expression of the gene or its ability to prevent or inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one 5 may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

Internalizing Proteins

The *tat* protein sequence (from HIV) can be used to internalize proteins into a 10 cell. See, e.g., Falwell *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91:664-68. For example, an 11 amino acid sequence (Y-G-R-K-K-R-R-Q-R-R; SEQ ID NO: 20) of the HIV *tat* protein (termed the "protein transduction domain," or TAT PDT) has been described as mediating delivery across the cytoplasmic membrane and the nuclear membrane of a cell. See Schwarze *et al.*, 1999, *Science* 285:1569-72; and Nagahara 15 *et al.*, 1998, *Nat. Med.* 4:1449-52. In these procedures, FITC-constructs (FITC-labeled G-G-G-G-Y-G-R-K-K-R-R-Q-R-R-R; SEQ ID NO: 21), which penetrate tissues following intraperitoneal administration, are prepared, and the binding of such constructs to cells is detected by fluorescence-activated cell sorting (FACS) analysis. Cells treated with a *tat*- β -gal fusion protein will demonstrate β -gal activity. 20 Following injection, expression of such a construct can be detected in a number of tissues, including liver, kidney, lung, heart, and brain tissue. It is believed that such constructs undergo some degree of unfolding in order to enter the cell, and as such, may require a refolding following entry into the cell.

It will thus be appreciated that the *tat* protein sequence may be used to 25 internalize a desired polypeptide into a cell. For example, using the *tat* protein sequence, a B7-L antagonist (such as an anti-B7-L selective binding agent, small molecule, soluble receptor, or antisense oligonucleotide) can be administered intracellularly to inhibit the activity of a B7-L molecule. As used herein, the term "B7-L molecule" refers to both B7-L nucleic acid molecules and B7-L polypeptides 30 as defined herein. Where desired, the B7-L protein itself may also be internally administered to a cell using these procedures. See also, Straus, 1999, *Science* 285:1466-67.

Cell Source Identification Using B7-L Polypeptide

In accordance with certain embodiments of the invention, it may be useful to be able to determine the source of a certain cell type associated with a B7-L polypeptide. For example, it may be useful to determine the origin of a disease or pathological condition as an aid in selecting an appropriate therapy. In certain 5 embodiments, nucleic acids encoding a B7-L polypeptide can be used as a probe to identify cells described herein by screening the nucleic acids of the cells with such a probe. In other embodiments, one may use anti-B7-L polypeptide antibodies to test for the presence of B7-L polypeptide in cells, and thus, determine if such cells are of the types described herein.

10

B7-L Polypeptide Compositions and Administration

Therapeutic compositions are within the scope of the present invention. Such B7-L polypeptide pharmaceutical compositions may comprise a therapeutically effective amount of a B7-L polypeptide or a B7-L nucleic acid molecule in admixture 15 with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Pharmaceutical compositions may comprise a therapeutically effective amount of one or more B7-L polypeptide selective binding agents in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

20

Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

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The pharmaceutical composition may contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrans), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents,

hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide),
5 solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), toxicity enhancing agents (such as alkali metal halides—preferably sodium or potassium chloride—or mannitol sorbitol), delivery vehicles,
10 diluents, excipients and/or pharmaceutical adjuvants. See *Remington's Pharmaceutical Sciences* (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990.

The optimal pharmaceutical composition will be determined by a skilled artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage. See, e.g., *Remington's Pharmaceutical Sciences, supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the B7-L molecule.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection may be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise
20 Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute. In one embodiment of the present invention, B7-L polypeptide compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized
25 cake or an aqueous solution. Further, the B7-L polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.
30

The B7-L polypeptide pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such

pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable, aqueous solution comprising the desired B7-L molecule in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a B7-L molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, B7-L polypeptide may be formulated as a dry powder for inhalation. B7-L polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Pub. No. WO 94/20069, which describes the pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, B7-L polypeptides that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the B7-L polypeptide. Diluents, flavorings, low melting point waxes, vegetable

oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an effective quantity of B7-L polypeptides in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional B7-L polypeptide pharmaceutical compositions will be evident to those skilled in the art, including formulations involving B7-L polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See, e.g., PCT/US93/00829, which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions.

Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, poly lactides (U.S. Patent No. 3,773,919 and European Patent No. 058481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-56), poly(2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-105), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent No. 133988). Sustained-release compositions may also include liposomes, which can be prepared by any of several methods known in the art. See, e.g., Eppstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-92; and European Patent Nos. 036676, 088046, and 143949.

The B7-L pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration may be stored in

lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in
5 sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for
10 producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

The effective amount of a B7-L pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives.
15 One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the B7-L molecule is being used, the route of administration, and the size (body weight, body surface, or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of
20 administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 µg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 µg/kg up to about 100 mg/kg; or 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to about 100 mg/kg.

25 The frequency of dosing will depend upon the pharmacokinetic parameters of the B7-L molecule in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over
30 time, or as a continuous infusion via an implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, e.g., orally; through injection by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes; by sustained release systems; or by 5 implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or other appropriate material onto which the desired molecule has been absorbed or encapsulated. Where an implantation device 10 is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

In some cases, it may be desirable to use B7-L polypeptide pharmaceutical compositions in an *ex vivo* manner. In such instances, cells, tissues, or organs that 15 have been removed from the patient are exposed to B7-L polypeptide pharmaceutical compositions after which the cells, tissues, or organs are subsequently implanted back into the patient.

In other cases, a B7-L polypeptide can be delivered by implanting certain cells 20 that have been genetically engineered, using methods such as those described herein, to express and secrete the B7-L polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The 25 encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

As discussed herein, it may be desirable to treat isolated cell populations (such 30 as stem cells, lymphocytes, red blood cells, chondrocytes, neurons, and the like) with one or more B7-L polypeptides. This can be accomplished by exposing the isolated cells to the polypeptide directly, where it is in a form that is permeable to the cell membrane.

Additional embodiments of the present invention relate to cells and methods (e.g., homologous recombination and/or other recombinant production methods) for

both the *in vitro* production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy. Homologous and other recombination methods may be used to modify a cell that contains a normally transcriptionally-silent B7-L gene, or an under-expressed gene, and thereby 5 produce a cell which expresses therapeutically efficacious amounts of B7-L polypeptides.

Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes. Kucherlapati, 1989, *Prog. in Nucl. Acid Res. & Mol. Biol.* 36:301. The basic technique was 10 developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas *et al.*, 1986, *Cell* 44:419-28; Thomas and Capecchi, 1987, *Cell* 51:503-12; Doetschman *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:8583-87) or to correct specific mutations within defective genes (Doetschman *et al.*, 1987, *Nature* 330:576-78). Exemplary homologous recombination techniques are 15 described in U.S. Patent No. 5,272,071; European Patent Nos. 9193051 and 505500; PCT/US90/07642, and PCT Pub No. WO 91/09955).

Through homologous recombination, the DNA sequence to be inserted into the genome can be directed to a specific region of the gene of interest by attaching it to targeting DNA. The targeting DNA is a nucleotide sequence that is complementary 20 (homologous) to a region of the genomic DNA. Small pieces of targeting DNA that are complementary to a specific region of the genome are put in contact with the parental strand during the DNA replication process. It is a general property of DNA that has been inserted into a cell to hybridize, and therefore, recombine with other pieces of endogenous DNA through shared homologous regions. If this 25 complementary strand is attached to an oligonucleotide that contains a mutation or a different sequence or an additional nucleotide, it too is incorporated into the newly synthesized strand as a result of the recombination. As a result of the proofreading function, it is possible for the new sequence of DNA to serve as the template. Thus, the transferred DNA is incorporated into the genome.

Attached to these pieces of targeting DNA are regions of DNA that may 30 interact with or control the expression of a B7-L polypeptide, e.g., flanking sequences. For example, a promoter/enhancer element, a suppressor, or an 'exogenous' transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding

the desired B7-L polypeptide. The control element controls a portion of the DNA present in the host cell genome. Thus, the expression of the desired B7-L polypeptide may be achieved not by transfection of DNA that encodes the B7-L gene itself, but rather by the use of targeting DNA (containing regions of homology with the 5 endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene sequence with recognizable signals for transcription of a B7-L gene.

In an exemplary method, the expression of a desired targeted gene in a cell (i.e., a desired endogenous cellular gene) is altered via homologous recombination into the cellular genome at a preselected site, by the introduction of DNA that 10 includes at least a regulatory sequence, an exon, and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit (in which the regulatory sequence, the exon, and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of 15 these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at 20 physiologically significant levels in the cell as obtained. The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

One method by which homologous recombination can be used to increase, or 25 cause, B7-L polypeptide production from a cell's endogenous B7-L gene involves first using homologous recombination to place a recombination sequence from a site-specific recombination system (e.g., Cre/loxP, FLP/FRT) (Sauer, 1994, *Curr. Opin. Biotechnol.*, 5:521-27; Sauer, 1993, *Methods Enzymol.*, 225:890-900) upstream of 30 (i.e., 5' to) the cell's endogenous genomic B7-L polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic B7-L polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the

recombination site located just upstream of the genomic B7-L polypeptide coding region in the cell line (Baubonis and Sauer, 1993, *Nucleic Acids Res.* 21:2025-29; O'Gorman *et al.*, 1991, *Science* 251:1351-55). Any flanking sequences known to increase transcription (e.g., enhancer/promoter, intron, translational enhancer), if 5 properly positioned in this plasmid, would integrate in such a manner as to create a new or modified transcriptional unit resulting in *de novo* or increased B7-L polypeptide production from the cell's endogenous B7-L gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just upstream of the cell's endogenous genomic B7-L polypeptide coding region is to use homologous recombination to introduce a second 10 recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, and translocation) (Sauer, 1994, *Curr. Opin. Biotechnol.*, 5:521-27; Sauer, 1993, *Methods Enzymol.*, 225:890-900) that would 15 create a new or modified transcriptional unit resulting in *de novo* or increased B7-L polypeptide production from the cell's endogenous B7-L gene.

An additional approach for increasing, or causing, the expression of B7-L polypeptide from a cell's endogenous B7-L gene involves increasing, or causing, the 20 expression of a gene or genes (e.g., transcription factors) and/or decreasing the expression of a gene or genes (e.g., transcriptional repressors) in a manner which results in *de novo* or increased B7-L polypeptide production from the cell's endogenous B7-L gene. This method includes the introduction of a non-naturally occurring polypeptide (e.g., a polypeptide comprising a site specific DNA binding 25 domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased B7-L polypeptide production from the cell's endogenous B7-L gene results.

The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, 30 (c) an exon, and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a) - (d) into a target gene in a cell such that the elements (b) - (d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the

integration of elements (a) - (f) such that the elements of (b) - (f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

If the sequence of a particular gene is known, such as the nucleic acid sequence of B7-L polypeptide presented herein, a piece of DNA that is complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece of DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding a B7-L polypeptide, which nucleotides may be used as targeting sequences.

B7-L polypeptide cell therapy, e.g., the implantation of cells producing B7-L polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a biologically active form of B7-L polypeptide. Such B7-L polypeptide-producing cells can be cells that are natural producers of B7-L polypeptides or may be recombinant cells whose ability to produce B7-L polypeptides has been augmented by transformation with a gene encoding the desired B7-L polypeptide or with a gene augmenting the expression of B7-L polypeptide. Such a modification may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered a B7-L polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing B7-L polypeptide be of human origin and produce human B7-L polypeptide. Likewise, it is preferred that the recombinant cells producing B7-L polypeptide be transformed with an expression vector containing a gene encoding a human B7-L polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow the

release of B7-L polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce B7-L polypeptides *ex vivo*, may be implanted directly into the patient without such encapsulation.

5 Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge *et al.* (PCT Pub. No. WO 95/05452 and PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are
10 biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down-regulation *in vivo* upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In
15 addition, see U.S. Patent Nos. 4,892,538; 5,011,472; and 5,106,627. A system for encapsulating living cells is described in PCT Pub. No. WO 91/10425 (Aebischer *et al.*). See also, PCT Pub. No. WO 91/10470 (Aebischer *et al.*); Wim *et al.*, 1991, *Exper. Neurol.* 113:322-29; Aebischer *et al.*, 1991, *Exper. Neurol.* 111:269-75; and Tresco *et al.*, 1992, *ASAIO* 38:17-23.

20 *In vivo* and *in vitro* gene therapy delivery of B7-L polypeptides is also envisioned. One example of a gene therapy technique is to use the B7-L gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding a B7-L polypeptide that may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct." The promoter may be homologous or heterologous to the
25 endogenous B7-L gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include DNA molecules designed for site-specific integration (e.g., endogenous sequences useful for homologous recombination), tissue-specific promoters, enhancers or silencers, DNA molecules capable of providing a selective
30 advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for example, for cell targeting), cell-specific internalization factors, transcription factors enhancing expression from a vector, and factors enabling vector production.

A gene therapy DNA construct can then be introduced into cells (either *ex*

vivo or *in vivo*) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein. Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other 5 vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

In yet other embodiments, regulatory elements can be included for the controlled expression of the B7-L gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of initiating a biological process, such as a DNA-binding protein or transcriptional activation protein (see PCT Pub. Nos. WO 96/41865, WO 97/31898, and WO 97/31899). The dimerization of the 10 proteins can be used to initiate transcription of the transgene.

An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain that results in the retention of the aggregated protein in the 20 endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (e.g., small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. See Aridor *et al.*, 2000, *Science* 287:816-17 and Rivera *et al.*, 2000, *Science* 25 287:826-30.

Other suitable control means or gene switches include, but are not limited to, the systems described herein. Mifepristone (RU486) is used as a progesterone antagonist. The binding of a modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two 30 transcription factors that then pass into the nucleus to bind DNA. The ligand-binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. Patent No. 5,364,791 and PCT Pub. Nos. WO 96/40911 and WO 97/10337.

Yet another control system uses ecdysone (a fruit fly steroid hormone), which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain, 5 DNA-binding domain, and ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. Patent No. 5,514,578 and PCT Pub. Nos. WO 97/38117, WO 96/37609, and WO 93/03162.

Another control means uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated 10 tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, i.e., it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758, 5,650,298, and 5,654,168.

Additional expression control systems and nucleic acid constructs are 15 described in U.S. Patent Nos. 5,741,679 and 5,834,186, to Inovir Laboratories Inc.

In vivo gene therapy may be accomplished by introducing the gene encoding B7-L polypeptide into cells via local injection of a B7-L nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. Hefti 1994, *Neurobiology* 25:1418-35. For example, a nucleic acid molecule encoding a B7-L polypeptide may 20 be contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (see, e.g., Johnson, PCT Pub. No. WO 95/34670; PCT App. No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence encoding a B7-L polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, 25 adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 30 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells that have been treated *in vitro* to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent Nos. 5,631,236

(involving adenoviral vectors), 5,672,510 (involving retroviral vectors), 5,635,399 (involving retroviral vectors expressing cytokines).

- Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (e.g., gene gun). Gene therapy materials and methods may also include inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cellspecific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent Nos. 4,970,154 (involving electroporation techniques), 5,679,559 (describing a lipoprotein-containing system for gene delivery), 5,676,954 (involving liposome carriers), 5,593,875 (describing methods for calcium phosphate transfection), and 4,945,050 (describing a process wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells), and PCT Pub. No. WO 96/40958 (involving nuclear ligands).

It is also contemplated that B7-L gene therapy or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). Such cells may be separately introduced into the patient, or the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A means to increase endogenous B7-L polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the B7-L polypeptide promoter, where the enhancer elements can serve to increase transcriptional activity of the B7-L gene. The enhancer elements used will be selected based on the tissue in which one desires to activate the gene - enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a gene encoding a B7-L polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the B7-L polypeptide promoter (and

optionally, inserted into a vector and/or 5' and/or 3' flanking sequences) using standard cloning techniques. This construct, known as a "homologous recombination construct," can then be introduced into the desired cells either *ex vivo* or *in vivo*.

Gene therapy also can be used to decrease B7-L polypeptide expression by 5 modifying the nucleotide sequence of the endogenous promoter. Such modification is typically accomplished via homologous recombination methods. For example, a DNA molecule containing all or a portion of the promoter of the B7-L gene selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example, the TATA box and/or the binding site of a 10 transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the corresponding B7-L gene. The deletion of the TATA box or the transcription activator binding site in the promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the B7-L 15 polypeptide promoter (from the same or a related species as the B7-L gene to be regulated) in which one or more of the TATA box and/or transcriptional activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. This construct, which also will 20 typically contain at least about 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified, may be introduced into the appropriate cells (either *ex vivo* or *in vivo*) either directly or via a viral vector as described herein. Typically, the integration of 25 the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

Therapeutic Uses

30 B7-L nucleic acid molecules, polypeptides, and agonists and antagonists thereof can be used to treat, diagnose, ameliorate, or prevent a number of diseases, disorders, or conditions, including those recited herein.

B7-L polypeptide agonists and antagonists include those molecules which regulate B7-L polypeptide activity and either increase or decrease at least one activity

of the mature form of the B7-L polypeptide. Agonists or antagonists may be co-factors, such as a protein, peptide, carbohydrate, lipid, or small molecular weight molecule, which interact with B7-L polypeptide and thereby regulate its activity. Potential polypeptide agonists or antagonists include antibodies that react with either 5 soluble or membrane-bound forms of B7-L polypeptides that comprise part or all of the extracellular domains of the said proteins. Molecules that regulate B7-L polypeptide expression typically include nucleic acids encoding B7-L polypeptide that can act as anti-sense regulators of expression.

Since transgenic mice expressing a related member of the B7 family showed 10 seminal vesicle hyperplasia (co-pending U.S. Patent App. No. 09/729,264, filed November 28, 2000), B7-L polypeptide agonists and antagonists may be useful in the treatment of reproductive disorders and proliferative disorders.

The overexpression of B7-L polypeptide may play a role in the growth and maintenance of cancer cells by causing seminal vesicle hyperplasia. Accordingly, 15 agonists or antagonists to B7-L polypeptide may be useful for the diagnosis or treatment of cancer. Examples of such cancers include, but are not limited to, seminal vesicle cancer, lung cancer, brain cancer, breast cancer, cancers of the hematopoietic system, prostate cancer, ovarian cancer, and testicular cancer. Other cancers are encompassed within the scope of the invention.

20 The overexpression of B7-L polypeptide may play a role in the inappropriate proliferation of cells by causing seminal vesicle hyperplasia. B7-L polypeptide may play a role in the inappropriate proliferation of cells based on overexpression causing seminal vesicle hyperplasia. Accordingly, agonists or antagonists to B7-L polypeptide may be useful for the diagnosis or treatment of diseases associated with 25 abnormal cell proliferation. Examples of such diseases include, but are not limited to, arteriosclerosis and vascular restenosis. Other diseases influenced by the inappropriate proliferation of cells are encompassed within the scope of the invention.

The overexpression of B7-L polypeptide may play a role in the reproductive system by causing seminal vesicle hyperplasia. Accordingly, agonists or antagonists 30 to B7-L polypeptide may be useful for the diagnosis or treatment of diseases associated with the reproductive system. Examples of such diseases include, but are not limited to, infertility, miscarriage, pre-term labor and delivery, and endometriosis. Other diseases of the reproductive system are encompassed within the scope of the invention.

Preferably, the B7-L nucleic acid molecules, polypeptides, and agonists and antagonists of the present invention are used to treat, diagnose, ameliorate, or prevent diseases associated with T-cell function (*e.g.*, functioning as a T-cell receptor decoy). For example, antibodies, soluble proteins comprising extracellular domains, or other regulators of B7-L polypeptide that result in prolonged or enhanced T-cell activation can be used to increased the immune response to tumors.

The B7-L nucleic acid molecules, polypeptides, and agonists and antagonists of the present invention may be used in the treatment of autoimmune disease, graft survival, immune cell activation for inhibiting tumor cell growth, T-cell dependent B-cell mediated diseases, and cancer gene immunotherapy. In one embodiment, antagonists or inhibitors of B7-L polypeptide function may be beneficial to alleviate symptoms in diseases with chronic immune cell dysfunction. Autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, immune thrombocytopenic purpura (ITP), and psoriasis, may be treated with antagonists or inhibitors of B7-L polypeptide. In addition, chronic inflammatory diseases, such as inflammatory bowel disease (Crohn's disease and ulcerative colitis), Grave's disease, Hashimoto's thyroiditis, and diabetes mellitus, may also be treated with inhibitors to B7-L polypeptide.

Antagonists of B7-L polypeptide may be used as immunosuppressive agents for bone marrow and organ transplantation and may be used to prolong graft survival. Such antagonists may provide significant advantages over existing treatments. Bone marrow and organ transplantation therapy must contend with T-cell mediated rejection of the foreign cells or tissue by the host. Present therapeutic regimens for inhibiting T-cell mediated rejection involve treatment with the drugs cyclosporine or FK506. While drugs are effective, patients suffer from serious side effects, including hepatotoxicity, nephrotoxicity, and neurotoxicity. The target for the cyclosporin/FK506 class of therapeutics is calcineurin, a phosphatase with ubiquitous expression. Inhibitors of B7-L polypeptide may lack the severe side effects observed with the use of the present immunotherapeutic agents. Antagonists of B7-L polypeptide may be used as immunosuppressive agents for autoimmune disorders, such as rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes, and systemic lupus erythematosus. Antagonists of the B7-L polypeptide may also be used to alleviate toxic shock syndrome, inflammatory bowel disease, allosensitization due to blood

transfusions, T-cell dependent B-cell mediated diseases, and the treatment of graft versus host disease.

Gene therapy using B7-L genes of the invention may be used in cancer immunotherapy. B7-L genes introduced into cancer cells can transform them into antigen presenting cells that can be recognized by the T-cells of the immune system when introduced back into an animal. Recognition of the transfected tumor cells by the T-cells results in the eradication of tumors expressing and tumors not expressing the B7-L gene. This immunotherapy approach may be used for various leukemias, sarcomas, melanomas, adenocarcinomas, breast carcinomas, prostate tumors, lung carcinomas, colon carcinomas, and other tumors. This invention encompasses using the B7-L gene in a similar manner to enhance T-cell activation in response to variety of tumors.

Many vaccines act by eliciting an effective and specific antibody response. Some vaccines, especially those against intestinal microorganisms (e.g., Hepatitis A virus and *Salmonella*), elicit a short-lived antibody response. It is desirable to potentiate and prolong this response in order to increase the effectiveness of the vaccine. Therefore, soluble B7-L polypeptides may serve as vaccine adjuvants.

Anti-viral responses may also be enhanced by activators or agonists of the B7-L polypeptide pathway. The enhancement of cellular immune functions by B7-L polypeptide/Fc fusion proteins may also be beneficial in eliminating virus-infected cells. In a complementary fashion, B7-L polypeptide/Fc fusion proteins may also have effects on humoral immune functions that may enhance antibody mediated responses and that may function to help clear free virus from the body.

Conversely, there are a number of clinical conditions that would be ameliorated by the inhibition of antibody production. Hypersensitivity is a normally beneficial immune response that is exaggerated or inappropriate, and leads to inflammatory reactions and tissue damage. Hypersensitivity reactions that are antibody-mediated may be particularly susceptible to antagonism by inhibitors of B7-L polypeptide activity. Allergies, hay fever, asthma, and acute edema cause type I hypersensitivity reactions, and these reactions may be suppressed by protein, antibody, or small molecule inhibitors of B7-L polypeptide activity.

Diseases that cause antibody-mediated hypersensitivity reactions, including systemic lupus erythematosus, arthritis (rheumatoid arthritis, reactive arthritis, and psoriatic arthritis), nephropathies (glomerulo-nephritis, membranous,

mesangiocapillary, focal segmental, focal necrotizing, crescentic, and proliferative tubulopathies), skin disorders (pemphigus, pemphigoid, and erythema nodosum), endocrinopathies (thyroiditis, Grave's, Hashimoto's, insulin-dependent diabetes mellitus), various pneumopathies (especially extrinsic alveolitis), various 5 vasculopathies, coeliac disease, with aberrant production of IgA, many anemias and thrombocytopenias, Guillain-Barre Syndrome, and myasthenia gravis, may be treated with B7-L polypeptide antagonists.

In addition, lymphoproliferative disorders, such as multiple myeloma, Waldenstrom's macroglobulinemia, and crioglobulinemias, may be inhibited by 10 protein, antibody, or small molecule antagonists of B7-L polypeptide.

Finally, graft versus host disease, an "artificial" immune disorder, may benefit from the inhibition of antibody production by B7-L polypeptide antagonists.

Agonists or antagonists of B7-L polypeptide function may be used (simultaneously or sequentially) in combination with one or more cytokines, growth 15 factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the condition being treated.

Other diseases or disorders caused by or mediated by undesirable levels of B7-L polypeptides are encompassed within the scope of the invention. Undesirable levels include excessive levels of B7-L polypeptides and sub-normal levels of B7-L 20 polypeptides.

Uses of B7-L Nucleic Acids and Polypeptides

Nucleic acid molecules of the invention (including those that do not themselves encode biologically active polypeptides) may be used to map the locations 25 of the B7-L gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization.

B7-L nucleic acid molecules (including those that do not themselves encode biologically active polypeptides), may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of a B7-L nucleic 30 acid molecule in mammalian tissue or bodily fluid samples.

Other methods may also be employed where it is desirable to inhibit the activity of one or more B7-L polypeptides. Such inhibition may be effected by nucleic acid molecules that are complementary to and hybridize to expression control sequences (triple helix formation) or to B7-L mRNA. For example, antisense DNA

or RNA molecules, which have a sequence that is complementary to at least a portion of a B7-L gene can be introduced into the cell. Anti-sense probes may be designed by available techniques using the sequence of the B7-L gene disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each 5 selected B7-L gene. When the antisense molecule then hybridizes to the corresponding B7-L mRNA, translation of this mRNA is prevented or reduced. Anti-sense inhibitors provide information relating to the decrease or absence of a B7-L polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative 10 inhibitor of one or more B7-L polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected B7-L polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

15 In addition, a B7-L polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to a B7-L polypeptide (as described herein) may be used for *in vivo* and *in vitro* diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of B7-L 20 polypeptide in a body fluid or cell sample. The antibodies may also be used to prevent, treat, or diagnose a number of diseases and disorders, including those recited herein. The antibodies may bind to a B7-L polypeptide so as to diminish or block at least one activity characteristic of a B7-L polypeptide, or may bind to a polypeptide to increase at least one activity characteristic of a B7-L polypeptide (including by 25 increasing the pharmacokinetics of the B7-L polypeptide).

The B7-L polypeptides of the present invention can be used to clone B7-L 30 polypeptide receptors, using an expression cloning strategy. Radiolabeled (125-Iodine) B7-L polypeptide or affinity/activity-tagged B7-L polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or cell line or tissue that expresses B7-L polypeptide receptors. RNA isolated from such cells or tissues can be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (such as COS or 293 cells) to create an expression library. A radiolabeled or tagged B7-L polypeptide can then be used as an affinity ligand to identify and isolate from this library the

subset of cells that express the B7-L polypeptide receptors on their surface. DNA can then be isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing B7-L polypeptide receptors is many-fold higher than in the original library. This 5 enrichment process can be repeated iteratively until a single recombinant clone containing a B7-L polypeptide receptor is isolated. Isolation of the B7-L polypeptide receptors is useful for identifying or developing novel agonists and antagonists of the B7-L polypeptide signaling pathway. Such agonists and antagonists include soluble B7-L polypeptide receptors, anti-B7-L polypeptide receptor antibodies, small 10 molecules, or antisense oligonucleotides, and they may be used for treating, preventing, or diagnosing one or more of the diseases or disorders described herein.

The murine and human B7-L nucleic acids of the present invention are also useful tools for isolating the corresponding chromosomal B7-L polypeptide genes. For example, mouse chromosomal DNA containing B7-L sequences can be used to 15 construct knockout mice, thereby permitting an examination of the *in vivo* role for B7-L polypeptide. The human B7-L genomic DNA can be used to identify heritable tissue-degenerating diseases.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

20

Example 1: Cloning of the Human B7-L Polypeptide Genes

Generally, materials and methods as described in Sambrook *et al. supra* were used to clone and analyze the genes encoding human and murine B7-L polypeptides.

A search of the Genbank-EMBL database was performed using the 25 TBLASTX program (<http://blast.wustl.edu>) and B7-H1 as the query sequence. A human genomic BAC clone was identified (Genbank accession no. AC080312) as containing a nucleic acid sequence encoding a member of the B7 family.

Plasmid DNA from various cDNA libraries was used as a template in PCR amplifications performed with the primers 2434-29 (5'-G-G-G-A-G-G-A-T-G-G-A- 30 A-T-C-C-T-G-A-G-C-3'; SEQ ID NO: 22) and 2434-34 (5'-C-T-G-G-T-A-T-G-C-T-G-A-A-G-G-C-T-C-C-3'; SEQ ID NO: 23). The PCR primers were designed to correspond to sequences within a putative exon in the BAC clone identified above. PCR amplifications were performed using standard techniques.

The expected 314 bp PCR fragment was obtained from cDNA libraries

generated from human fetal pancreas, bladder, kidney, liver, ovary, scalp, gall bladder, uterus, T-lymphocytes, and trachea. In addition, the expected fragment was obtained from a number of tumor cell lines, including prostate tumor T1175, ovary tumor T23, lung tumor T27, breast tumor T1543, and a breast carcinoma cell line.

5 The 314 bp fragment was also obtained from several sub-pools of human mixed-tissue cDNA libraries (each sub-pool containing approximately 15,000 clones). A custom synthesized library optimized for full-length cDNA clones – the LTI-FL cDNA library (Life Technologies Inc.) – was used for further cloning experiments.

The 314 bp fragment obtained in PCR amplifications of human mixed-tissue cDNA libraries was isolated and cloned into the pCR2.1-TOPO® vector (Invitrogen, Carlsbad, CA). The DNA sequence of a selected clone was determined to confirm that the sequence of the clone was identical to that of the originally identified genomic sequence. The 314 bp fragment was then excised from the vector by Eco RI digestion and labeled by incorporation of ³²P-dCTP. The labeled fragment was used to screen 150,000 bacterial colonies derived from a 15,000-clone pool of the LTI-FL cDNA library that tested positive in prior PCR amplification experiments. Colonies were transferred from LB/ampicillin plates to nitrocellulose filters, pre-hybridized in 6X SSC, 0.5% SDS, 1X Denhardt's solution and 100 µg/ml denatured salmon sperm DNA for 3 hours at 60°C. Following the addition of 1 x 10⁶ cpm/ml of the ³²P-labeled probe, hybridization was performed overnight under the same conditions. Filters were washed twice for 30 minutes at room temperature in 2X SSC and 0.1% SDS and then twice for 30 minutes at 65°C in 0.1X SSC and 0.1% SDS. Filters were then exposed to X-ray film overnight at -80°C with intensifying screens.

A single positive colony was identified in this manner and plasmid DNA from this clone was prepared by standard methods. The cDNA insert from this colony was 2.6 kb in length. DNA sequence analysis confirmed that the clone contained the putative coding region of the B7-L gene. However, although the sequence of the cDNA clone was closely related to the AL080312 genomic sequence, it was not identical.

30 Sequence analysis of the full-length cDNA for human B7-L polypeptide indicated that the gene comprises a 846 bp open reading frame encoding a protein of 282 amino acids and possessing a potential signal peptide of 24 amino acids in length at its amino-terminus (Figure 1; predicted signal peptide indicated by underline).

The predicted protein product of the B7-L gene is related to the B7 family of proteins. These proteins are members of the immunoglobulin superfamily and function as regulators of the T-cell mediated immune response. The members of this family possess amino acid sequences that are poorly conserved, and thus difficult to 5 distinguish from other related molecules using computational methods. However, while the members of the B7 family of proteins share a lower level of sequence identity, the members of this family exhibit several shared structural features. These 10 structural features include the alignment of certain cysteine residues (which form disulfide bonds within the immunoglobulin domains), the overall size of the molecule, the location of the transmembrane domain within the polypeptide, and a small cytoplasmic domain and an extracellular region that contain immunoglobulin V (variable) and C (constant) domains. The members of the B7 family are Type-1 15 membrane proteins.

The known members of the B7 family include CD80 (B7-1), CD86 (B7-2), 20 B7-rp1, and B7-H1. B7-1 and B7-2 interact with CD28 and CTLA-4 and are mediators of the T cell costimulatory pathway. B7-rp1 binds to a distinct receptor (ICOS; inducible co-stimulator) and is also a stimulator of T-cell proliferation. B7-H1 also co-stimulates T cell proliferation, but does not bind CD28, CTLA-4, or 25 ICOS. Other proteins exhibiting sequence homology to the B7 family include the butyrophilins and PRO352. Still more distantly related are the myelin oligodendrocyte proteins (MOGs). Figures 2A-2C illustrate an amino acid sequence alignment of the human proteins B7-L polypeptide, CD80 (B7-1), CD86 (B7-2), B7-H1, B7rp-1, PRO352, butyrophilin BTF1, butyrophilin BTF2, butyrophilin BTF4, butyrophilin BTF3, and butyrophilin.

A comparison of the B7-L nucleic sequence with a genomic sequence 30 database (Celera, Rockville, MD) yielded several good matches, allowing the determination of a tentative intron-exon structure for the B7-L gene. A contig was built around each putative exon from genomic sequence tags (GSTs) identified in the genomic sequence database. Currently, there is no overlap between the contigs, so whether this assembly represents the true genomic sequence cannot yet be confirmed. Figures 3A-3E illustrate the genomic nucleotide sequence for human B7-L polypeptide. The location of the exons (underline) and deduced amino acid sequence of the exons are indicated. The upstream boundary of exon 1 is yet to be determined.

Example 2: B7-L mRNA Expression

The expression of B7-L mRNA is examined by Northern blot analysis. Multiple human tissue northern blots (Clontech) are probed with a suitable restriction fragment isolated from a human B7-L polypeptide cDNA clone. The probe is labeled 5 with ^{32}P -dCTP using standard techniques.

Northern blots are prehybridized for 2 hours at 42°C in hybridization solution (5X SSC, 50% deionized formamide, 5X Denhardt's solution, 0.5% SDS, and 100 mg/ml denatured salmon sperm DNA) and then hybridized at 42°C overnight in fresh hybridization solution containing 5 ng/ml of the labeled probe. Following 10 hybridization, the filters are washed twice for 10 minutes at room temperature in 2X SSC and 0.1% SDS, and then twice for 30 minutes at 65°C in 0.1X SSC and 0.1% SDS. The blots are then exposed to autoradiography.

The expression of B7-L mRNA is localized by *in situ* hybridization. A panel 15 of normal embryonic and adult mouse tissues is fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm . Sectioned tissues are permeabilized in 0.2 M HCl, digested with Proteinase K, and acetylated with triethanolamine and acetic anhydride. Sections are prehybridized for 1 hour at 60°C in hybridization 20 solution (300 mM NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1X Denhardt's solution, 0.2% SDS, 10 mM DTT, 0.25 mg/ml tRNA, 25 $\mu\text{g}/\text{ml}$ polyA, 25 $\mu\text{g}/\text{ml}$ polyC and 50% formamide) and then hybridized overnight at 60°C in the same 25 solution containing 10% dextran and 2×10^4 cpm/ μl of a ^{33}P -labeled antisense riboprobe complementary to the human B7-L gene. The riboprobe is obtained by *in vitro* transcription of a clone containing human B7-L cDNA sequences using standard techniques.

Following hybridization, sections are rinsed in hybridization solution, treated with RNaseA to digest unhybridized probe, and then washed in 0.1X SSC at 55°C for 30 minutes. Sections are then immersed in NTB-2 emulsion (Kodak, Rochester, NY), exposed for 3 weeks at 4°C, developed, and counterstained with hematoxylin and 30 eosin. Tissue morphology and hybridization signal are simultaneously analyzed by darkfield and standard illumination for brain (one sagittal and two coronal sections), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, proximal colon, and distal colon), pituitary, liver, lung, heart, spleen, thymus, lymph nodes, kidney, adrenal, bladder, pancreas, salivary gland, male and female reproductive

organs (ovary, oviduct, and uterus in the female; and testis, epididymus, prostate, seminal vesicle, and vas deferens in the male), BAT and WAT (subcutaneous, perirenal), bone (femur), skin, breast, and skeletal muscle.

5 Example 3: Production of B7-L Polypeptides

A. Expression of B7-L Polypeptides in Bacteria

PCR is used to amplify template DNA sequences encoding a B7-L polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow 10 for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary vector, such as pAMG21 (ATCC no. 98113) containing the lux promoter and a gene encoding kanamycin resistance is digested with Bam HI and Nde I for directional cloning of inserted DNA. The ligated mixture is transformed into an *E. coli* host 15 strain by electroporation and transformants are selected for kanamycin resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm the presence of the insert.

Transformed host cells are incubated in 2xYT medium containing 30 µg/mL kanamycin at 30°C prior to induction. Gene expression is induced by the addition of 20 N-(3-oxohexanoyl)-dl-homoserine lactone to a final concentration of 30 ng/mL followed by incubation at either 30°C or 37°C for six hours. The expression of B7-L polypeptide is evaluated by centrifugation of the culture, resuspension and lysis of the bacterial pellets, and analysis of host cell proteins by SDS-polyacrylamide gel electrophoresis.

25 Inclusion bodies containing B7-L polypeptide are purified as follows. Bacterial cells are pelleted by centrifugation and resuspended in water. The cell suspension is lysed by sonication and pelleted by centrifugation at 195,000 xg for 5 to 10 minutes. The supernatant is discarded, and the pellet is washed and transferred to a homogenizer. The pellet is homogenized in 5 mL of a Percoll solution (75% liquid 30 Percoll and 0.15 M NaCl) until uniformly suspended and then diluted and centrifuged at 21,600 xg for 30 minutes. Gradient fractions containing the inclusion bodies are recovered and pooled. The isolated inclusion bodies are analyzed by SDS-PAGE.

A single band on an SDS polyacrylamide gel corresponding to *E. coli*-produced B7-L polypeptide is excised from the gel, and the N-terminal amino acid

sequence is determined essentially as described by Matsudaira *et al.*, 1987, *J. Biol. Chem.* 262:10-35.

B. Expression of B7-L Polypeptide in Mammalian Cells

5 PCR is used to amplify template DNA sequences encoding a B7-L polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary 10 expression vector, pCEP4 (Invitrogen, Carlsbad, CA), that contains an Epstein-Barr virus origin of replication, may be used for the expression of B7-L polypeptides in 293-EBNA-1 cells. Amplified and gel purified PCR products are ligated into pCEP4 vector and introduced into 293-EBNA cells by lipofection. The transfected cells are selected in 100 µg/mL hygromycin and the resulting drug-resistant cultures are grown 15 to confluence. The cells are then cultured in serum-free media for 72 hours. The conditioned media is removed and B7-L polypeptide expression is analyzed by SDS-PAGE.

20 B7-L polypeptide expression may be detected by silver staining. Alternatively, B7-L polypeptide is produced as a fusion protein with an epitope tag, such as an IgG constant domain or a FLAG epitope, which may be detected by 25 Western blot analysis using antibodies to the peptide tag.

B7-L polypeptides may be excised from an SDS-polyacrylamide gel, or B7-L fusion proteins are purified by affinity chromatography to the epitope tag, and subjected to N-terminal amino acid sequence analysis as described herein.

25

C. Expression and Purification of B7-L Polypeptide in Mammalian Cells

B7-L polypeptide expression constructs are introduced into 293 EBNA or CHO cells using either a lipofection or calcium phosphate protocol.

To conduct functional studies on the B7-L polypeptides that are produced, 30 large quantities of conditioned media are generated from a pool of hygromycin selected 293 EBNA clones. The cells are cultured in 500 cm² Nunc Triple Flasks to 80% confluence before switching to serum free media a week prior to harvesting the media. Conditioned media is harvested and frozen at -20°C until purification.

Conditioned media is purified by affinity chromatography as described below. The media is thawed and then passed through a 0.2 µm filter. A Protein G column is equilibrated with PBS at pH 7.0, and then loaded with the filtered media. The column is washed with PBS until the absorbance at A_{280} reaches a baseline. B7-L polypeptide 5 is eluted from the column with 0.1 M Glycine-HCl at pH 2.7 and immediately neutralized with 1 M Tris-HCl at pH 8.5. Fractions containing B7-L polypeptide are pooled, dialyzed in PBS, and stored at -70°C.

For Factor Xa cleavage of the human B7-L polypeptide-Fc fusion polypeptide, affinity chromatography-purified protein is dialyzed in 50 mM Tris-HCl, 10 100 mM NaCl, 2 mM CaCl₂ at pH 8.0. The restriction protease Factor Xa is added to the dialyzed protein at 1/100 (w/w) and the sample digested overnight at room temperature.

Example 4: Production of Anti-B7-L Polypeptide Antibodies

15 Antibodies to B7-L polypeptides may be obtained by immunization with purified protein or with B7-L peptides produced by biological or chemical synthesis. Suitable procedures for generating antibodies include those described in Hudson and Bay, *Practical Immunology* (2nd ed., Blackwell Scientific Publications).

In one procedure for the production of antibodies, animals (typically mice or 20 rabbits) are injected with a B7-L antigen (such as a B7-L polypeptide), and those with sufficient serum titer levels as determined by ELISA are selected for hybridoma production. Spleens of immunized animals are collected and prepared as single cell suspensions from which splenocytes are recovered. The splenocytes are fused to mouse myeloma cells (such as Sp2/0-Ag14 cells), are first incubated in DMEM with 25 200 U/mL penicillin, 200 µg/mL streptomycin sulfate, and 4 mM glutamine, and are then incubated in HAT selection medium (hypoxanthine, aminopterin, and thymidine). After selection, the tissue culture supernatants are taken from each fusion well and tested for anti-B7-L antibody production by ELISA.

Alternative procedures for obtaining anti-B7-L antibodies may also be 30 employed, such as the immunization of transgenic mice harboring human Ig loci for production of human antibodies, and the screening of synthetic antibody libraries, such as those generated by mutagenesis of an antibody variable domain.

Example 5: Expression of B7-L Polypeptide in Transgenic Mice

To assess the biological activity of B7-L polypeptide, a construct encoding a B7-L polypeptide/Fc fusion protein under the control of a liver specific ApoE promoter is prepared. The delivery of this construct is expected to cause pathological changes that are informative as to the function of B7-L polypeptide. Similarly, a 5 construct containing the full-length B7-L polypeptide under the control of the beta actin promoter is prepared. The delivery of this construct is expected to result in ubiquitous expression.

To generate these constructs, PCR is used to amplify template DNA sequences encoding a B7-L polypeptide using primers that correspond to the 5' and 3' ends of 10 the desired sequence and which incorporate restriction enzyme sites to permit insertion of the amplified product into an expression vector. Following amplification, PCR products are gel purified, digested with the appropriate restriction enzymes, and ligated into an expression vector using standard recombinant DNA techniques. For example, amplified B7-L polypeptide sequences can be cloned into an expression 15 vector under the control of the human β -actin promoter as described by Graham *et al.*, 1997, *Nature Genetics*, 17:272-74 and Ray *et al.*, 1991, *Genes Dev.* 5:2265-73.

Following ligation, reaction mixtures are used to transform an *E. coli* host strain by electroporation and transformants are selected for drug resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm 20 the presence of an appropriate insert and absence of mutation. The B7-L polypeptide expression vector is purified through two rounds of CsCl density gradient centrifugation, cleaved with a suitable restriction enzyme, and the linearized fragment containing the B7-L polypeptide transgene is purified by gel electrophoresis. The purified fragment is resuspended in 5 mM Tris, pH 7.4, and 0.2 mM EDTA at a 25 concentration of 2 mg/mL.

Single-cell embryos from BDF1 x BDF1 bred mice are injected as described (PCT Pub. No. WO 97/23614). Embryos are cultured overnight in a CO₂ incubator and 15-20 two-cell embryos are transferred to the oviducts of a pseudopregnant CD1 female mice. Offspring obtained from the implantation of microinjected embryos are 30 screened by PCR amplification of the integrated transgene in genomic DNA samples as follows. Ear pieces are digested in 20 mL ear buffer (20 mM Tris, pH 8.0, 10 mM EDTA, 0.5% SDS, and 500 mg/mL proteinase K) at 55°C overnight. The sample is then diluted with 200 mL of TE, and 2 mL of the ear sample is used in a PCR reaction using appropriate primers.

At 8 weeks of age, transgenic founder animals and control animals are sacrificed for necropsy and pathological analysis. Portions of spleen are removed and total cellular RNA isolated from the spleens using the Total RNA Extraction Kit (Qiagen) and transgene expression determined by RT-PCR. RNA recovered from 5 spleens is converted to cDNA using the SuperScript™ Preamplification System (Gibco-BRL) as follows. A suitable primer, located in the expression vector sequence and 3' to the B7-L polypeptide transgene, is used to prime cDNA synthesis from the transgene transcripts. Ten mg of total spleen RNA from transgenic founders and controls is incubated with 1 mM of primer for 10 minutes at 70°C and placed on ice. 10 The reaction is then supplemented with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 10 mM of each dNTP, 0.1 mM DTT, and 200 U of SuperScript II reverse transcriptase. Following incubation for 50 minutes at 42°C, the reaction is stopped by heating for 15 minutes at 72°C and digested with 2U of RNase H for 20 minutes at 37°C. Samples are then amplified by PCR using primers specific for B7-L 15 polypeptide.

Example 6: Biological Activity of B7-L Polypeptide in Transgenic Mice

Prior to euthanasia, transgenic animals are weighed, anesthetized by isofluorane and blood drawn by cardiac puncture. The samples are subjected to 20 hematology and serum chemistry analysis. Radiography is performed after terminal exsanguination. Upon gross dissection, major visceral organs are subject to weight analysis.

Following gross dissection, tissues (*i.e.*, liver, spleen, pancreas, stomach, the entire gastrointestinal tract, kidney, reproductive organs, skin and mammary glands, 25 bone, brain, heart, lung, thymus, trachea, esophagus, thyroid, adrenals, urinary bladder, lymph nodes and skeletal muscle) are removed and fixed in 10% buffered Zn-Formalin for histological examination. After fixation, the tissues are processed into paraffin blocks, and 3 mm sections are obtained. All sections are stained with hematoxylin and exosin, and are then subjected to histological analysis.

30 The spleen, lymph node, and Peyer's patches of both the transgenic and the control mice are subjected to immunohistology analysis with B cell and T cell specific antibodies as follows. The formalin fixed paraffin embedded sections are deparaffinized and hydrated in deionized water. The sections are quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and

incubated in rat monoclonal anti-mouse B220 and CD3 (Harlan, Indianapolis, IN). Antibody binding is detected by biotinylated rabbit anti-rat immunoglobulins and peroxidase conjugated streptavidin (BioGenex, San Ramon, CA) with DAB as a chromagen (BioTek, Santa Barbara, CA). Sections are counterstained with 5 hematoxylin.

After necropsy, MLN and sections of spleen and thymus from transgenic animals and control littermates are removed. Single cell suspensions are prepared by gently grinding the tissues with the flat end of a syringe against the bottom of a 100 mm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ). Cells are washed 10 twice, counted, and approximately 1×10^6 cells from each tissue are then incubated for 10 minutes with 0.5 µg CD16/32(FcγIII/II) Fc block in a 20 µL volume. Samples are then stained for 30 minutes at 2-8°C in a 100 µL volume of PBS (lacking Ca⁺ and Mg⁺), 0.1% bovine serum albumin, and 0.01% sodium azide with 0.5 µg antibody of FITC or PE-conjugated monoclonal antibodies against CD90.2 (Thy-1.2), CD45R 15 (B220), CD11b (Mac-1), Gr-1, CD4, or CD8 (PharMingen, San Diego, CA). Following antibody binding, the cells are washed and then analyzed by flow cytometry on a FACScan (Becton Dickinson).

While the present invention has been described in terms of the preferred 20 embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - 5 (a) the nucleotide sequence as set forth in SEQ ID NO: 1;
 - (b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2;
 - (c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of either (b) or (c); and
 - 10 (d) a nucleotide sequence complementary to either (b) or (c).
2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding a polypeptide which is at least about 15 70 percent identical to the polypeptide as set forth in SEQ ID NO: 2, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
 - (b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1 or (a);
 - (c) a region of the nucleotide sequence of SEQ ID NO: 1, (a), or (b) 20 encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide fragment has an activity of the encoded polypeptide as set forth in SEQ ID NO: 2, or is antigenic;
 - (d) a region of the nucleotide sequence of SEQ ID NO: 1 or any of (a)-
(c) comprising a fragment of at least about 16 nucleotides;
 - 25 (e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d); and
 - (f) a nucleotide sequence complementary to any of (a)-(d).
3. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;

- (b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 5 (c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 10 (e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 15 (f) a nucleotide sequence of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;
- (g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (f); and
- (h) a nucleotide sequence complementary to any of (a) - (e).
- 20 4. A vector comprising the nucleic acid molecule of any of Claims 1, 2, or 3.
5. A host cell comprising the vector of Claim 4.
- 25 6. The host cell of Claim 5 that is a eukaryotic cell.
7. The host cell of Claim 5 that is a prokaryotic cell.
- 30 8. A process of producing a B7-L polypeptide comprising culturing the host cell of Claim 5 under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.
9. A polypeptide produced by the process of Claim 8.

10. The process of Claim 8, wherein the nucleic acid molecule comprises promoter DNA other than the promoter DNA for the native B7-L polypeptide operatively linked to the DNA encoding the B7-L polypeptide.

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11. The isolated nucleic acid molecule according to Claim 2, wherein the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

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12. A process for determining whether a compound inhibits B7-L polypeptide activity or B7-L polypeptide production comprising exposing a cell according to any of Claims 5, 6, or 7 to the compound and measuring B7-L polypeptide activity or B7-L polypeptide production in said cell.

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13. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2.

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14. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence as set forth in SEQ ID NO: 3, optionally further comprising an amino-terminal methionine;
- (b) an amino acid sequence for an ortholog of SEQ ID NO: 2;
- (c) an amino acid sequence which is at least about 70 percent identical to the amino acid sequence of SEQ ID NO: 2, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 comprising at least about 25 amino acid residues, wherein the fragment has an activity of the polypeptide set forth in SEQ ID NO: 2, or is antigenic; and
- (e) an amino acid sequence for an allelic variant or splice variant of the amino acid sequence as set forth in SEQ ID NO: 2 or any of (a)-(c).

15. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 5 (b) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- (c) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2;
- 10 (d) the amino acid sequence as set forth in SEQ ID NO: 2 which has a C-and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2; and
- (e) the amino acid sequence as set forth in SEQ ID NO: 2 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in SEQ 15 ID NO: 2.

16. An isolated polypeptide encoded by the nucleic acid molecule of any 20 of Claims 1, 2, or 3, wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2.

17. The isolated polypeptide according to Claim 14, wherein the percent identity is determined using a computer program selected from the group consisting 25 of GAP, BLASTP, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

18. A selective binding agent or fragment thereof that specifically binds the polypeptide of any of Claims 13, 14, or 15.

30 19. The selective binding agent or fragment thereof of Claim 18 that specifically binds the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2, or a fragment thereof.

20. The selective binding agent of Claim 18 that is an antibody or fragment thereof.

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21. The selective binding agent of Claim 18 that is a humanized antibody.

22. The selective binding agent of Claim 18 that is a human antibody or fragment thereof.

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23. The selective binding agent of Claim 18 that is a polyclonal antibody or fragment thereof.

24. The selective binding agent of Claim 18 that is a monoclonal antibody or fragment thereof.

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25. The selective binding agent of Claim 18 that is a chimeric antibody or fragment thereof.

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26. The selective binding agent of Claim 18 that is a CDR-grafted antibody or fragment thereof.

27. The selective binding agent of Claim 18 that is an antiidiotypic antibody or fragment thereof.

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28. The selective binding agent of Claim 18 that is a variable region fragment.

29. The variable region fragment of Claim 28 that is a Fab or a Fab' fragment.

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30. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for a polypeptide having the amino acid sequence of SEQ ID NO; 2.

31. The selective binding agent of Claim 18 that is bound to a detectable label.

32. The selective binding agent of Claim 18 that antagonizes B7-L
5 polypeptide biological activity.

33. A method for treating, preventing, or ameliorating a B7-L polypeptide-related disease, condition, or disorder comprising administering to a patient an effective amount of a selective binding agent according to Claim 18.

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34. A selective binding agent produced by immunizing an animal with a polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

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35. A hybridoma that produces a selective binding agent capable of binding a polypeptide according to any of Claims 1, 2, or 3.

36. A method of detecting or quantitating the amount of B7-L polypeptide using the anti-B7-L antibody or fragment of Claim 18.

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37. A composition comprising the polypeptide of any of Claims 13, 14, or 15, and a pharmaceutically acceptable formulation agent.

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38. The composition of Claim 37, wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or antioxidant.

39. The composition of Claim 37 wherein the polypeptide comprises the amino acid sequence as set forth in SEQ ID NO: 3.

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40. A polypeptide comprising a derivative of the polypeptide of any of Claims 13, 14, or 15.

41. The polypeptide of Claim 40 that is covalently modified with a water-soluble polymer.

42. The polypeptide of Claim 41, wherein the water-soluble polymer is selected from the group consisting of polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols, and polyvinyl alcohol.

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43. A composition comprising a nucleic acid molecule of any of Claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

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44. The composition of Claim 43, wherein said nucleic acid molecule is contained in a viral vector.

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45. A viral vector comprising a nucleic acid molecule of any of Claims 1, 2, or 3.

46. A fusion polypeptide comprising the polypeptide of any of Claims 13, 14, or 15 fused to a heterologous amino acid sequence.

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47. The fusion polypeptide of Claim 46, wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

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48. A method for treating, preventing, or ameliorating a medical condition comprising administering to a patient the polypeptide of any of Claims 13, 14, or 15, or the polypeptide encoded by the nucleic acid of any of Claims 1, 2, or 3.

49. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

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(a) determining the presence or amount of expression of the polypeptide of any of Claims 13, 14, or 15, or the polypeptide encoded by the nucleic acid molecule of any of Claims 1, 2, or 3 in a sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

50. A device, comprising:
- a membrane suitable for implantation; and
 - cells encapsulated within said membrane, wherein said cells secrete a protein of any of Claims 13, 14, or 15; and
- 5 said membrane is permeable to said protein and impermeable to materials detrimental to said cells.
51. A method of identifying a compound which binds to a B7-L polypeptide comprising:
- contacting the polypeptide of any of Claims 13, 14, or 15 with a compound; and
 - determining the extent of binding of the B7-L polypeptide to the compound.
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52. The method of Claim 51, further comprising determining the activity of the polypeptide when bound to the compound.
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53. A method of modulating levels of a polypeptide in an animal comprising administering to the animal the nucleic acid molecule of any of Claims 1, 2, or 3.
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54. A transgenic non-human mammal comprising the nucleic acid molecule of any of Claims 1, 2, or 3.
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55. A process for determining whether a compound inhibits B7-L polypeptide activity or B7-L polypeptide production comprising exposing a transgenic mammal according to Claim 54 to the compound, and measuring B7-L polypeptide activity or B7-L polypeptide production in said mammal.
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56. A nucleic acid molecule of any of Claims 1, 2, or 3 attached to a solid support.

57. An array of nucleic acid molecules comprising at least one nucleic acid molecule of any of Claims 1, 2, or 3.

58. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 2 with at least one conservative amino acid substitution selected from the group consisting of: methionine at position 4; leucine or methionine at position 12; leucine or valine at position 13; leucine or valine at position 16; leucine or valine at position 17; leucine, valine, or methionine at position 18; leucine or valine at position 23; leucine at position 26; leucine or valine at position 27; valine or leucine at position 31; leucine or isoleucine at position 39; alanine at position 46; valine at position 48; alanine at position 52; isoleucine at position 54; glutamic acid at position 61; valine at position 64; glutamic acid at position 66; leucine, methionine, or valine at position 67; valine or leucine at position 69; arginine at position 73; leucine at position 76; valine at position 79; methionine at position 80; tyrosine at position 83; arginine at position 84; aspartic acid at position 85; arginine at position 87; glutamic acid at position 88; aspartic acid at position 92; tyrosine at position 97; lysine at position 98; leucine, isoleucine, or methionine at position 103; isoleucine, leucine, or methionine at position 108; isoleucine at position 115; isoleucine at position 117; leucine or isoleucine at position 120; valine or isoleucine at position 122; serine at position 123; glutamic acid at position 124; serine at position 127; phenylalanine at position 128; arginine at position 129; phenylalanine at position 131; valine at position 132; alanine at position 137; valine or isoleucine at position 143; alanine at position 148; glycine at position 149; isoleucine or methionine at position 155; isoleucine or methionine at position 157; isoleucine at position 166; tyrosine at position 174; isoleucine, leucine, or methionine at position 179; isoleucine at position 180; leucine at position 194; phenylalanine at position 215; serine at position 218; serine at position 222; isoleucine or leucine at position 226; valine or leucine at position 227; leucine or valine at position 231; isoleucine at position 240; aspartic acid at position 242; methionine or leucine at position 245; arginine at position 246; threonine at position 256; valine or isoleucine at position 260; leucine or isoleucine at position 262; leucine or valine at position 268; valine or methionine at position 272; valine at position 273; valine, isoleucine, or methionine at position 275; phenylalanine at position 278; valine or isoleucine at position 279; isoleucine or

valine at position 281; and arginine at position 282; wherein the polypeptide has an activity of the polypeptide set forth in SEQ ID NO: 2.

FIG. 1A

gcggcagctc cactcagccca gtacccagat acgctggaa cttccccag cc atg gct	58
<u>Met Ala</u>	<u>1</u>
tcc ctg ggg cag atc ctc ttc tgg agc ata att agc atc atc att att	106
<u>Ser Leu Gly Gln Ile Leu Phe Trp Ser Ile Ile Ser Ile Ile Ile Ile</u>	
5 10 15	
ctg gct gga gca att gca ctc atc att ggc ttt ggt att tca ggg aga	154
<u>Leu Ala Gly Ala Ile Ala Leu Ile Ile Gly Phe Gly Ile Ser Gly Arg</u>	
20 25 30	
cac tcc atc aca gtc act act gtc gcc tca gct ggg aac att ggg gag	202
<u>His Ser Ile Thr Val Thr Val Ala Ser Ala Gly Asn Ile Gly Glu</u>	
35 40 45 50	
gat gga atc ctg agc tgc act ttt gaa cct gac atc aaa ctt tct gat	250
<u>Asp Gly Ile Leu Ser Cys Thr Phe Glu Pro Asp Ile Lys Leu Ser Asp</u>	
55 60 65	
atc gtg ata caa tgg ctg aag gaa ggt gtt tta ggc ttg gtc cat gag	298
<u>Ile Val Ile Gln Trp Leu Lys Glu Gly Val Leu Gly Leu Val His Glu</u>	
70 75 80	
ttc aaa gaa ggc aaa gat gag ctg tcg gag cag gat gaa atg ttc aga	346
<u>Phe Lys Glu Gly Lys Asp Glu Leu Ser Glu Gln Asp Glu Met Phe Arg</u>	
85 90 95	
ggc cgg aca gca gtg ttt gct gat caa gtg ata gtt ggc aat gcc tct	394
<u>Gly Arg Thr Ala Val Phe Ala Asp Gln Val Ile Val Gly Asn Ala Ser</u>	
100 105 110	
ttg cgg ctg aaa aac gtg caa ctc aca gat gct ggc acc tac aaa tgt	442
<u>Ieu Arg Leu Lys Asn Val Gln Leu Thr Asp Ala Gly Thr Tyr Lys Cys</u>	
115 120 125 130	
tat atc atc act tot aaa ggc aag ggg aat gct aac ctt gag tat aaa	490
<u>Tyr Ile Ile Thr Ser Lys Gly Lys Asn Ala Asn Leu Glu Tyr Lys</u>	
135 140 145	
act gga gcc ttc agc atg ccg gaa gtg aat gtg gac tat aat gcc agc	538
<u>Thr Gly Ala Phe Ser Met Pro Glu Val Asn Val Asp Tyr Asn Ala Ser</u>	
150 155 160	
tca gag acc ttg cgg tgt gag gct ccc cga tgg ttc ccc cag ccc aca	586
<u>Ser Glu Thr Leu Arg Cys Glu Ala Pro Arg Trp Phe Pro Gln Pro Thr</u>	
165 170 175	
gtg gtc tgg gca tcc caa gtt gac cag gga gcc aac ttc tcg gaa gtc	634
<u>Val Val Trp Ala Ser Gln Val Asp Gln Gly Ala Asn Phe Ser Glu Val</u>	
180 185 190	

FIG. 1B

tcc aat acc agc ttt gag ctg aac tct gag aat gtg acc atg aag gtt 682
 Ser Asn Thr Ser Phe Glu Leu Asn Ser Glu Asn Val Thr Met Lys Val
 195 200 205 210

gtg tct gtg ctc tac aat gtt acg atc aac aca tac tcc tgt atg 730
 Val Ser Val Leu Tyr Asn Val Thr Ile Asn Asn Thr Tyr Ser Cys Met
 215 220 225

att gaa aat gac att gcc aaa gca aca ggg gat atc aac gtg aca gaa 778
 Ile Glu Asn Asp Ile Ala Lys Ala Thr Gly Asp Ile Lys Val Thr Glu
 230 235 240

tcg qag atc aaa agg cgg agt cac cta cag ctg cta aac tca aag gct 826
 Ser Glu Ile Lys Arg Arg Ser His Leu Gln Leu Asn Ser Lys Ala
 245 250 255

tct ctg tgt gtc tct tct ttc ttt gcc atc agc tgg gca ctt ctg cct 874
 Ser Leu Cys Val Ser Ser Phe Ala Ile Ser Trp Ala Leu Leu Pro
 260 265 270

ctc agc cct tac ctg atg cta aaa taa tggcccttgg ccacaaaaaa 921
 Leu Ser Pro Tyr Leu Met Leu Lys
 275 280

gcatgcaaag tcattgttac aacaggatc tacagaacta tttcaccacc agatatgacc 981
 tagtttata ttctggag gaaatgaatt catatctaga agtctggact gagcaaacaa 1041
 gagcaagaaa caaaaagaag cccaaagcag aaggctccaa tatgaacaag ataaatctat 1101
 cttcaaagac atattagaag ttggaaaaat aattcatgtg aactagacaa gtgtgttaag 1161
 agtgataagt aaaaatgcacg tggagacaa tgcatccoca gatctcaggg acctccccct 1221
 gcctgtcacc tggggagtga gaggacagga tagtgcatttgc tctttgtctc tgaattttta 1281
 gttatatgtg ctgtaatgtt gctctgagga agcccctgga aagtctatcc caacatatcc 1341
 acatcttata ttccacaaaataaagctgttag tatgttaccc aagacgctgc taattgactg 1401
 ccacttcgca actcaggggc ggctgcattt tagtaatggg tcaaatgatt cacttttat 1461
 gatgcttcca aagggtgcctt ggcttctctt cccaaactgac aaatgccaaa gttgagaaaa 1521
 atgatcataa ttttagcata aacagagcag tcggcgacac cgatttata aataaactga 1581
 gcaccttctt tttaaacaataa caaatgcggg tttatttctc agatgatgtt catccgtgaa 1641
 tggccaggg aaggaccttt caccttgact atatggcatt atgtcatcac aagctctgag 1701
 gcttcttctt tecatcctgc gtggacagct aagacctcag tttcaatag catctagagc 1761
 agtgggactc agctggggtg atttgcggg ccatctccgg gggatgtct gaagacaatt 1821

FIG. 1C

ttggttacct caatgaggga gtggaggagg atacagtgtc actaccaact agtggataaa 1881
ggccagggat gctgtcaac ctccattaccat gtacaggacg tctccccatt acaactaccc 1941
aatccgaagt gtcaactgtg tcaggactaa gaaaccctgg ttttagtag aaaaaggcct 2001
gaaaagaggg gagccaacaa atctgtctgc ttccatcacat tagtcattgg caaataagca 2061
ttctgtctct ttggctgctg cctcagcaca gagagccaga actctatcg gcaccaggat 2121
aacatctctc agtgaacaga gttgacaagg cctatggaa atgcctgtatgg gattatctt 2181
cagcttgttg agcttctaag ttctttccc ttcattctac cctgcaagcc aagttctgtta 2241
agagaaaatgc ctgagttcta gtcaggttt tcttactctg aatttagatc tccagaccct 2301
tccctggccac aattcaaatt aaggcaacaa acatataacct tccatgaagc acacacagac 2361
tttgaaagc aaggacaatg actgcttcaa ttgaggcctt gaggaatgaa gcttgaagg 2421
aaaagaatac ttgtttcca gcccccttcc cacactcttc atgtgttaac cactgccttc 2481
ctggacccttg gagccacggt gactgttatta catgttgtta tagaaaactg attttaragt 2541
tctgatcggtt caagagaatg attaaatata catttcctaa aaaaaaaaaa aaaaaaaaaa 2601
aa 2603

FIG. 2A

20	40	60	
Agp1-51578 : MASESQIILFWSTLISIHLLAGAYALI..TG.FG..TSERHSITTTVASAENIGEDCID			: 54
Cd80_Human : MGHTRRQGTSPSKCPYENPFOLVLAG..LSH..EC..SEV..IHV..TKE..VKEVATH			: 48
Cd86_Human : ..VGLSNILFVMAFL..SCAAPLKKQ..SFYFNETAQD			: 32
B7-H1_Huma : MRIFAVFETM..TYWHR..LNS..PTVTVEDKLYTV..EYGSNMT..I			: 38
B7rp-1_Hum : SRLGSPG.....LIFLLESSSLRADTQEKEVR..EMVGSQVER			: 35
Pro352_Hum : MERRRGSPGMGVHGAAKGAIAWFC..LTGAELOVPEDEV..LVGTEATL			: 48
Btf1_Human : MESAAABHFSRPA...LILLILISTCAL..VSQOHIVVGETDPI..STVGENNTI			: 49
Btsf2a2_Hu : MEPAAAAHFSLPASHLILLLSICAI..VSQOFIVVGPANPDE..AMVGENNTI			: 53
Btf4_Human : FHVSLILVQLITPC..SAOPSVIIGPSGPIL..AMVGEDSDI			: 37
Btn3a3_Hum :MMASSLAFLILLNFBySFLFVQLLTPCSEQESVUGPSGPIL..AMVGEDADE			: 50
Btn_Human :MAVFPSSGIIPRCIUTIIIQLQPKLDSRFIDVIGPPEPIL..EVVGEDAEL			: 48
80	100	120	
Agp1-51578 : SCTFEETI..KISDI..VQWILKEGVLG..LVHEFKEGKDELSEQDEMFRGRJAFPA			: 105
Cd80_Human : S...GHNVSVEELAQTRGYMKXKEKKMVLT..WMS..GDM..NIWPENRAT..IF			: 93
Cd86_Human : PQQFANSQNOQISLELV..VFWGDOENLVN..EWYL..GKEKFDSVHSKIMGRI..SF			: 82
B7-H1_Huma : ECKFPVKEKOLDIAPL..IVYHEMEDKNN..QEVH..BEEDLKVCHSSYRQFARILK.			: 89
B7rp-1_Hum : SCACPGESRFQIADV..YVYVACTSSESITV..VTVYHIPPONSSLENVDSRPNPALES.P			: 88
Pro352_Hum : CCSFSBEPGFRQIAQL..NLIVCLIDIE..D1VHESPAG..QDAGSAMANRITALFP			: 97
Btf1_Human : RCHISBEK..NAEDY..EVRFPRSOFS..PAVFVYKGGRTEREEOMEETYRGRITTFVSK			: 101
Btsf2a2_Hu : RCHISBEK..NAEDY..EVRFPRSOFS..PAVFVYKGGRTEREEOMEETYRGRITTFVSK			: 105
Btf4_Human : PCHIPEPTM..SAFTV..EIKWVSSSLR..QVNVYADGKEVEDROSAPYRGRFTSLR			: 88
Btn3a3_Hum : PCHIPEPTM..SAFTV..EIKWVSSSLR..QVNVYADGKEVEDROSAPYRGRFTSLR			: 101
Btn_Human : PCHIPENA..SAFHL..EIKWFRKKVS..PAVLVHRDGREQAEQMPETYRGRAILVQ			: 99
140	160	180	
Agp1-51578 : DQIVGNASLRKKVQLADACTIKKWHITSK..C..KGN4NEYKTE..FSMPEINW..			: 157
Cd80_Human : D..ITNMLSIVHLAIRPSDEGTICKWVKYDQDAFKREHLAPTESVKGDFPTESDSDFE			: 151
Cd86_Human : C..SDSWTLRERHKLQDQDKGLVQCLIIHHKKPTGMRILHQMSSELVLSNFSQDPI			: 135
B7-H1_Huma : DQISLGNAAALCITDNKLQDAGVIROMISY..SGADM..KRTIVKVN..PYNKINO			: 139
B7rp-1_Hum : AGPLRGDFSLRLEKIVPODEOKSHCLVLSQSI..CFQEZLVSVENTLVAERNFSVTV..S			: 144
Pro352_Hum : DLIAQGNASLRLRVRVRADECGSTCIVFSIRDF..S..SEASLOVAPYSKESM..T			: 148
Btf1_Human : D..IHRGSVALVTHIRNITACENGTYRCYF..Q..ERS..YDE..IHLVVAGLGSKELISM..			: 153
Btsf2a2_Hu : D..INRGSVALVTHIRNITACENGTYRCYF..Q..GRS..YDE..IHLVVAGLGSKELIEI			: 157
Btf4_Human : DGTACKAALRHENVTAQSDSKLICYF..Q..GDF..YEKA..WELKVALGSQDLTEV			: 141
Btn3a3_Hum : DGTACKAALRHENVTAQSDSKLICYF..Q..GDF..YEKA..WELKVALGSQDLTEV..			: 154
Btn_Human : DGTAKGRVALRIRGVRYRSDDGEYTOEFR..ED..GS..YEEAIIUEKVAALGSDFEHISM..			: 152
200	220	240	
Agp1-51578 :D.YNASSETIIGCAAPRPEPQPT..UVRASQVDQEANFSEVSNTSFELNSENVTMKV			: 210
Cd80_Human : IPTSNIR.....RUCGSTSEGEP..HHSVLENGEELNAINWTV..SQDP			: 193
Cd86_Human : V..PISNITENVYENITESSINGTPEPKKGSVL..LTKNSTIEYDGIMQKSQDN			: 186
B7-H1_Huma : RILVVDPPVT..SEEHLITOA..EGELEKAE..WINTSSDRQVLSGKTTTINSK..R			: 186
B7rp-1_Hum : .APHSPSQ..DEUTFTSITTINGTFRPN..WIG..INKEDNSLLDQALQNDTVFLN			: 192
Pro352_Hum : LEPNKDLRPGDTVPTTSESYQGYPEAE..WVQDQGVPLTGNVTTASQ..MAN			: 197
Btf1_Human : ...RGHEDGGRUPOISRGWIKEL..TWRDP..YGGVIAL..KEVSM..D			: 196
Btsf2a2_Hu : ...KAQEDGSIWIZGIGSGGWYIPEL..TVWRDP..YGEWHA..KEVSTA..I			: 200
Btf4_Human : ...KGYEDGGIHLICRSTGTYPOBQ..IKHNSNA..KGENIPAV..EAPVVA..E			: 184
Btn3a3_Hum : ...KGYEDGGIHLICRSTGTYPOBQ..IKHNSDT..KGENIPAV..EAPVVA..E			: 197
Btn_Human :QVOENGECIHECTSVGVWPEPO..IOWRTS..KGEKFEST..SESRNP..D			: 195

FIG. 2B

	* 260 *	* 280 *	* 300 *	
Agp1-51578 : VSVLYNV	INNNYSCMIPNDI	AKATGDTKYTESETIKRRSHLOLLNSK	: 257	
Cd80_Human : ETELYAVSSKLDF	NMTTINHSFMCLIRY	GHLR	VNQFENWNNTTKOEHFPDNLLPS	: 246
Cd86_Human : VTELYDVTSISLSVSFPDVTSNMIFCILLET	DKTRILLSSPFISIBLEDPOPPPQBI	. R	: 241	
B7-H1_Huma : EBKLFENVITSLRINT	TNEIFYCTFRLDPEENHT	AELVIPELPLAB	: 233	
B7rp-1_Hum : MRCIYDVVSVLRIAR	EPSVNIGGCCHEVILQONILTVG9QTGNNDGERDKITENPVST	: 249		
Pro352_Hum : EOCIEIDVHEVIRVWL	GANGTYSCLVNPVLOQ	DAHXSVTITGQPMTF	: 244	
Btf1_Human : ADGLFMVTTAVIIRD	KVVRNMSCSINKTLIGOKKESVITLPESRMPSV	. SP	: 246	
Btsf2a2_Hu : ADGLFMVTTAVIIRD	KVVRNMSCSINKTLIGOKKESVITLPESRMPSA	. SB	: 250	
Btf4_Human : EVGLYEVAASVIMRG	GESEGVSCLLNSLCELEKTASTSIADPFRSA	. QH	: 234	
Btn3a3_Hum : GVELYAYAASVIMRG	SSGGGVSCIIURNSLGLEKTASTSIADPFRSA	. QH	: 247	
Btn_Human : EPGLEYTAASVIMRD	DSTKNVSCYIONILGQEKWVLIPTASSPLPR	. TP	: 245	
	* 320 *	* 340 *	* 360 *	
Agp1-51578 : ASILCVSSFFAISSWALPILSHYIMK				: 282
Cd80_Human : WAFTLISWNGIFVECCILTYCB	APR	C	RERRRNERL.RRRESVR.PV	: 288
Cd86_Human : W	ITAVIPTVLCWVYFCILLLWKKKKRPRNSYKCGTNMEREESEQTKKREKIHP			: 298
B7-H1_Huma : PPNERTHLWILGAEL	CLCVALITFL	FRERKGMMMDVKKCGIQLDTNSKKQSPTHLE	: 288	
B7rp-1_Hum : GEKNAATWSILAVICMIVVVVVAIAAGVC	RDRCLQHSYAGAWAVSPETEILTGHY	: 302		
Pro352_Hum : PPE	AIKVTVGLSCLTAAVIAAEVC	WRKIKQSC.BEENAGAEDQDGEGZGSKTAL	: 299	
Btf1_Human : CAVALEPITV	WV	MPIIAVCYWINKLOEK	: 276	
Btsf2a2_Hu : WIVVALAVLTASPVWSMIVILAVFICPMAVSICCIRKLOREKKILSGEKKVEQEKEITA				: 310
Btf4_Human : WTAAPAGTIPILLIILLAGASVFLWROOKEKIALS				: 263
Btn3a3_Hum : WTAALASTLPISSLILLAGASVFLWROOKEKIALS				: 305
Btn_Human : WIVAVAVIILMVGLLITRGSLIFTWA	IV	NERPABR	RNFSSK	: 286
	* 380 *	* 400 *	* 420 *	
Agp1-51578 :				:
Cd80_Human :				:
Cd86_Human : ERSDEAQRVFKSSKTSSCDKSDTCF				: 323
B7-H1_Huma : ST				: 290
B7rp-1_Hum :				:
Pro352_Hum : QPIKHSDSKEDDGQEIA				: 316
Btf1_Human :				:
Btsf2a2_Hu : QOFQOELKWRR				TFLHAADVVLDPDTAHPELFILSEDRRSVRRG : 352
Btf4_Human :				:
Btn3a3_Hum : EKLOEELKWRKIQYMARGEKSLAYHEWKMALFKPADVILDPTANAILLVSEDQRSVQRA				: 365
Btn_Human : BRILLEELKWKK				ATLHAVDVTLDPTAHPHLFLYEDSKSVRLE : 328
	* 440 *	* 460 *	* 480 *	
Agp1-51578 :				:
Cd80_Human :				:
Cd86_Human :				:
B7-H1_Huma :				:
B7rp-1_Hum :				:
Pro352_Hum :				:
Btf1_Human :				:
Btsf2a2_Hu : PYRQRVPDNPERFDQSOPCVLGWESFASGKHYWEVEVENVMVWTGVCRHSVE.RKGEVILL				: 411
Btf4_Human :				:
Btn3a3_Hum : EEPRLDLPDNPERFEWRYCVLGCENFTSGRHYWEVEVGRDRKEWHIGVCSKNVERKKGVVKM				: 425
Btn_Human : DSRQKLPEKTERFDSWPCVLGRETFTSGRHYWEVEVGDRDWAIGVCREN.MKKGFDPM				: 387

FIG. 2C

	* 500 *	* 520 *	* 540 *	
Agp1-51578 :				:
Cd80_Human :				-
Cd86_Human :				-
B7-H1_Huma :				-
B7rp-1_Hum :				-
Pro352_Hum :				-
Btf1_Human :				-
Btsf2a2_Hu :	IPQNGFWTLEMF.GNQYRALSSPERILPLKESLCRGGVFLDYAGDVSFYNMRDRSHIYT			: 470
Btf4_Human :				-
Btn3a3_Hum :	TPENGYWTMGLTDGNKYRALTEPRTNLKLPEPPRKVGIFLDYETGEISFYNAATDGSHIYT			: 495
Btn_Human :	TPENGFWAVELY.GNGYWALTPLRPLPIAGPPRRVGIFLDYESGDISFYNMNDGSDIYT			: 446
	* 560 *	* 580 *	* 600 *	
Agp1-51578 :				:
Cd80_Human :				-
Cd86_Human :				-
B7-H1_Huma :				-
B7rp-1_Hum :				-
Pro352_Hum :				-
Btf1_Human :				-
Btsf2a2_Hu :	CPRSAFTVPVRPFRL.GSDDSPIFICPALTGASGVMVPPEEGLKLHRVGTHQSL			: 523
Btf4_Human :				-
Btn3a3_Hum :	FPHASFSEPLYPVFRILTLLEPTALTICPIPKEVESSPDOPDLPDHSLETPLTPGLANESG			: 545
Btn_Human :	FSNVTFSGPLRFFCLWSSGKKPLTCPIADGPERVTVTIANAQDLIKEIPLSPMGEESAP			: 506
	* 620 *			
Agp1-51578 :				:
Cd80_Human :				-
Cd86_Human :				-
B7-H1_Huma :				-
B7rp-1_Hum :				-
Pro352_Hum :				-
Btf1_Human :				-
Btsf2a2_Hu :				-
Btf4_Human :				-
Btn3a3_Hum :	EPQAEVTSLLLPAHPGREVSPSATTNQNHHKLQARTEALY			: 584
Btn_Human :	RDADTLHSKLIPTQPSQGAP			: 526

FIG. 3A

ttatttgag attaaatctc cctccctaca gctcccttcc acagtcagga ggaaagtctt 60
 gctgaatgca ggcgcgttggaa caaggcccg ggaagtttt cctcgccacc ctccccaaagt 120
 caagacttgg aaaggcagct ctgagccct ggcttggctg gctggccgga gcaggcagcc 180
 actgtgcctg caggaaattc tgcacagcca gtttccat acctgagccg tctacagctg 240
 cacgcaccac tcccgccctc aacacactat ttäaggccaa tacacggag ctgggttgtga 300
 gtcaccaagg aaggcaggcgg cagtcact cagcactac ccagatacgt q 352

EXON 1

gqaaccttcc ccagcc atg gct tcc ctg ggg cag atc ctc ttc tgg ag 400
 Met Ala Ser Leu Gly Gln Ile Leu Phe Trp Ser

gtagtatata ctcttctctc tatccttatg ggtgaaggga acagcagtga ggtctttgg 460
 ggatgctgag cactgctggg ctgtccata ggaccccacc tgtaattta tctgatccag 520
 cattgggcca gcgggagccct ggccagacaa ggcctaact ggattgact taacatccca 580
 ctcagctgc tcaaggcacc tgttcagggt tagggagggt tttgggttgtt ggctgccan 640
 ggagggctgg cgcttgctgg gcagcttgg ctgangggga gtacgtgccaa agctggcat 700
 tccccnttag atgtcaaggc tgtgagcacc atggggacac tgagcaggct ggtgtgggtt 760
 geatggtggg atggcaggga atccatgact gegacagtgg agggatgca ggaggaccct 820
 ttatgcagcc cccagggggga cggaggagaa tgtgcttca ttctctgnng gtgtgatttc 880
 ttgctactct tgagcaattc acaccaaacac ccctccccag gccccccca ctaaaagggtt 940
 gggccctag tttccctagg agctccctgg agaggagaac ttgtctgtgc tgctgtgtct 1000
 gctgctgtg ctgctgtgc tgaactcccc agagaggaag aaggaagcaa gttccctccc 1060
 ctacctttg ctctccctc gagctacatt ctatgtccct ctttttccct cagaacagca 1120
 ttaccctcc tggnaaggaa agagatggaa agtgcagggt tttcttgaaa tgattgattc 1180
 agtgcagtc acaactcagg acagtgtta gggaggctga tacttgttgg ttntagagg 1240
 tgtttgaagg accagtcctc caattttttt cttttccca caaaaggaag aaaagaagag 1300
 agggaaaggag agagaaagg aatcctcagg tcaacagtga aaagttgtg aagagactac 1360
 gagagtaatg ttactttcca gaggcagcc agaaaggctt ttcccttggaa acaaaatatc 1420
 agctccctg actcgactca cgtttatctg aaatcacctg cccggtagca gaggtgaagg 1480
 actctgcagg gctgggtgggg gntggctgca gccggcacttc tgcccccacc cctgtgcctg 1540

FIG. 3B

gctacagcac tgtgctccag gagaaggcgt gggtggcagg ggcttccttc tagacccttc 1600
tgccctgaga ggatgtccct aagaagcacc tatgtcagag gagcttagat tttgtgagtg 1660
actccttccc tgccctgcac taggtgtctt agagcagegt gtccaccact gccttgtgaa 1720
gactcgttat cacctcagag aagttaaggc caggtgactg tgaaaactgtg catgagggga 1780
tcttgctctg aaatcccaag ctctctcccc ctgcacccct tgccctgct cctgccagtc 1840
ccagttctcc atgcccccca ttcttcaggc cttgatccca aggacatgaa acactctcc 1900
tgcaacaagt ggctccattg ttcagaggc agatggatg tttgggtctt ctgcataatgc 1960
tttgcatcca gtctgactgg ttttgctgga ggattagcag gaggtgggtg ttccaaaattc 2020
gtatccccta cccttcagaa acccaactggc atcaacagag actcctcttgc ttggctctg 2080
catgccttgt tagcttattca tgctctcagc ttctcagaagc agcactgtc cataagggtg 2140
gcagatcctt tgtcaaggc acctccagga agagggccaa tgagcagctt cagtggggag 2200
caggatcatg aaagctcatc tcaggatgc aggtggagcc tgctgggtt aactcttgac 2260
cctaataacc ctcttcctt gtcataatgg cottaattca taaaataacc tctcctggag 2320
gtgatcatga cagtggtgat gtggAACGC tcatgnnnnn nnnnnaccca aatgtccatc 2380
aatgataggc tggataaaaga aatgtggca cgtatacacc atagaataact atgcagccat 2440
aaaaaaagaat gagttcatgt cctttgcagg aacatggatg aagctggaaa ccacattct 2500
cagcaaacta acacagggac agaaaaccaa acaccatgt ttctcactca taaaatggag 2560
ttgaacaatg agaacacatg gatacaggaa ggggaacatc acacactggg gcctgtcagg 2620
ggggctgggg gcaaggggag ggagggcatt aggacaaata atgcacgtcag gtctaaaac 2680
ctagatgacg ggttgcattt tgcagcaac caccatgaca catgtgtacc tatgtaacgc 2740
acctgcacgt tctgcacatg tatcccgaaa cttaaagtaa aataaaataa aaaataattg 2800
gtgaggatta tatcaaaaaca cagtgggtt atttagatga tggattaca ggtgatttat 2860
ttgtotcatt ttgcattttt tatattttctt aaactttctg tagtaaatat gttttgttt 2920
ttgtttttt aataaaagaa aacagactcc cactgatgac ttacggagtg cagtagatga 2980
gtgtgtgagtg ggtacaaagt gaagaagatg tgaccattgt ccttaatggc gttaaagtc 3040
tatggaaagga gataagacag ggagacaaat gacacaagag agtggacagt gatgctcagg 3100
gaaggctggg aacaggacca gtccttcata agtggcacct ggcattaaag aatgttttc 3160

FIG. 3C

atcattcttg ccccaacagt ctccatatat ggtttccct gatctggaaa taggacagta 3220
 aatggcacag tcagaggcaa gggtaactt tttcattgtat cccagagccc cagggggatg 3280
 gggatggaat tgctgaaatt cctttctaag gtacccgtt ctagaaca gcttatgtgg 3340
 aaaaaaaaaatg tgagatgttt tatgttaactc atcttatcca caacagattt gtaccccgat 3400
 tgcaaaaggcag cacatcaaga ggaatggaga actaattttc tgtttcttc tctttcccc 3460
 tag c ata att agc atc atc att att ctg gct gga gca att gca ctc atc 3509
 Ile Ile Ser Ile Ile Ile Ile Leu Ala Gly Ala Ala Ile Ile Ile Ile

EXON 2

att ggc ttt ggt att tca q gtatgtat ttcttcatt actacaatct 3557
 Ile Gly Phe Gly Ile Ser Gly

attcaccctt caccattaaa ctcctaata ctcagtcgt tagcatggaa agagccaga 3617
 gctttgaccc agaaaacctc agagtatagc attcatattt ctacttctt tgctttctct 3677
 acattatgtca gtttaagtccct cgacttcgtt gtcctcattt tttattttt atttattttt 3737
 tttagacag ggtctggctc tggtgccag gttggagtgc agtggcatga tctcagctta 3797
 ctgcaacctc caccttcgtt gttcaagcca tcttcacc tcagectccc aagtagctag 3857
 gactacaggc atgtgcacca ccatgccagg ctaattttt tatttttagt agagatgggg 3917
 ntttcaccat gttgtcagg ctggctctaa actcctgagc tcaagcaatc cacctgcctc 3977
 ggcctccaa agtgcggaa ttgcggctt gagccacgtt gcccggctt gnnnnnnnnn 4037
 ngtatttta atttatcaca gaagtctctg tctggcttcc agcaatgaag ggtttgggtt 4097
 tagaaggccc aaggcttccc ttagcattga tctttgttcc ctgaaactgca g gg aga 4153
 Arg

cac tcc atc aca gtc act act gtc gcc tca gct ggg aac att ggg gag 4202
 His Ser Ile Thr Val Thr Val Ala Ser Ala Gly Asn Ile Gly Glu

gat gga atc ctg agc tgc act ttt gaa cct gac atc aaa ctt tct gat 4250
 Asp Gly Ile Leu Ser Cys Thr Phe Glu Pro Asp Ile Lys Leu Ser Asp

EXON 3

atc gtg ata caa tgg ctg aag gaa ggt gtt tta ggc ttg gtc cat gag 4298
 Ile Val Ile Gln Trp Leu Lys Glu Val Leu Gly Ile Val His Glu

ttc aaa gaa ggc aaa gat gag ctg tgc gag cag gat gaa atg tcc aga 4346
 Phe Lys Glu Gly Lys Asp Glu Leu Ser Glu Gln Asp Glu Met Phe Arg

ggc cg₂ aca gca gtg ttt gct gat caa gtg ata gtt ggc aat gcc tct 4394
 Gly Arg Thr Ala Val Phe Ala Asp Gln Val Ile Val Gly Asn Ala Ser

FIG. 3D

<u>ttt</u> <u>cgg</u> <u>ctg</u> <u>aaa</u> <u>aac</u> <u>gtt</u> <u>caa</u> <u>ctc</u> <u>aca</u> <u>gat</u> <u>qct</u> <u>qgc</u> <u>acc</u> <u>tac</u> <u>aaa</u> <u>tgt</u>	4442
Leu Arg Leu Lys Asn Val Gln Leu Thr Asp Ala Gly Thr Tyr Lys Cys	
<u>tat</u> <u>atc</u> <u>atc</u> <u>act</u> <u>tct</u> <u>aaa</u> <u>qgc</u> <u>aag</u> <u>qqq</u> <u>aat</u> <u>qct</u> <u>aac</u> <u>ctt</u> <u>qag</u> <u>tat</u> <u>aaa</u>	4490
Tyr Ile Ile Thr Ser Lys Gly Lys Asn Ala Asn Leu Glu Tyr Lys	
<u>act</u> <u>gga</u> <u>q</u> <u>gtgagttact</u> <u>tttggagaga</u> <u>gat</u> <u>tttttaa</u> <u>agcaccaaaaa</u> <u>gtat</u> <u>tttgagg</u>	4546
Thr Gly Ala	
<u>ctaa</u> <u>aggat</u> <u>ta</u> <u>tgagttgctt</u> <u>atgaaatat</u> <u>g</u> <u>tttgacccaa</u> <u>tatcagacaa</u> <u>tgtatgc</u> <u>tcc</u> <u>cc</u>	4606
<u>aatt</u> <u>ctgct</u> <u>a</u> <u>acagctgtt</u> <u>ttgc</u> <u>catttt</u> <u>atggccaaga</u> <u>ctcacacaa</u> <u>ccccaaa</u> <u>agt</u>	4666
<u>agccaa</u> <u>actat</u> <u>aagagaggcc</u> <u>ttaaaaatct</u> <u>tttgttctgg</u> <u>gttctctctg</u> <u>cttcaga</u> <u>attt</u>	4726
<u>tccaggtgt</u> <u>ttcttotattc</u> <u>aacaaaataa</u> <u>atgt</u> <u>agcc</u> <u>ctc</u> <u>ttttgtggta</u> <u>gccc</u> <u>ctggta</u>	4786
<u>cagctaataa</u> <u>gagcagcc</u> <u>ta</u> <u>gc</u> <u>ctgggaca</u> <u>tttccacg</u> <u>ct</u> <u>aagt</u> <u>tagaccc</u> <u>a</u> <u>gcgc</u> <u>caatc</u>	4846
<u>ctgaaat</u> <u>atcag</u> <u>tat</u> <u>ttcaga</u> <u>gactcat</u> <u>gac</u> <u>agt</u> <u>gagt</u> <u>gca</u> <u>ttt</u> <u>taga</u> <u>agt</u> <u>tc</u> <u>aaagg</u> <u>gaa</u>	4906
<u>ctaacc</u> <u>aaaa</u> <u>cga</u> <u>aaaacaac</u> <u>att</u> <u>gtc</u> <u>ctt</u> <u>aaact</u> <u>catta</u> <u>at</u> <u>nnnnnnnn</u> <u>nnaggaa</u> <u>aat</u>	4966
<u>gcctaatt</u> <u>cc</u> <u>ctaagg</u> <u>gata</u> <u>gg</u> <u>cttttta</u> <u>tgacc</u> <u>act</u> <u>ga</u> <u>ag</u> <u>ttc</u> <u>c</u> <u>t</u> <u>t</u> <u>ttt</u> <u>ggag</u> <u>aa</u>	5026
<u>aattt</u> <u>tctat</u> <u>tgtaacc</u> <u>ata</u> <u>ta</u> <u>cct</u> <u>tag</u> <u>aat</u> <u>ttt</u> <u>agg</u> <u>tc</u> <u>ct</u> <u>ttt</u> <u>ggg</u> <u>gtt</u> <u>gat</u> <u>ttgg</u> <u>cc</u>	5086
<u>aaagg</u> <u>gtt</u> <u>gt</u> <u>gat</u> <u>gtat</u> <u>agc</u> <u>aat</u> <u>actat</u> <u>tg</u> <u>g</u> <u>caaa</u> <u>ata</u> <u>att</u> <u>at</u> <u>tgc</u> <u>cac</u> <u>ct</u> <u>at</u> <u>aata</u> <u>ca</u> <u>at</u>	5146
<u>gggtat</u> <u>aca</u> <u>cagg</u> <u>tg</u> <u>acc</u> <u>tct</u> <u>gag</u> <u>ga</u> <u>g</u> <u>act</u> <u>g</u> <u>at</u> <u>gg</u> <u>tgg</u> <u>ctt</u> <u>ca</u> <u>at</u> <u>gt</u> <u>at</u> <u>aaaa</u> <u>aaaa</u>	5206
<u>aaaaaaaa</u> <u>at</u> <u>act</u> <u>gc</u> <u>attt</u> <u>ttaagg</u> <u>cc</u> <u>ta</u> <u>gc</u> <u>gtt</u> <u>cc</u> <u>aaa</u> <u>gg</u> <u>ctat</u> <u>attt</u> <u>tctt</u> <u>ac</u> <u>tgt</u> <u>gt</u>	5266
<u>ctgact</u> <u>tttgg</u> <u>cat</u> <u>ctg</u> <u>cc</u> <u>c</u> <u>tt</u> <u>ctg</u> <u>act</u> <u>ttt</u> <u>tgacc</u> <u>ct</u> <u>gca</u> <u>g</u> <u>cc</u> <u>ttc</u> <u>agc</u> <u>atg</u> <u>ccg</u>	5321
Phe Ser Met Pro	
<u>gaa</u> <u>gt</u> <u>g</u> <u>aat</u> <u>gt</u> <u>g</u> <u>ac</u> <u>t</u> <u>at</u> <u>at</u> <u>g</u> <u>cc</u> <u>ag</u> <u>t</u> <u>ca</u> <u>g</u> <u>aq</u> <u>ac</u> <u>tt</u> <u>cg</u> <u>q</u> <u>tg</u> <u>g</u> <u>q</u>	5370
Glu Val Asn Val Asp Tyr Asn Ala Ser Ser Glu Thr Leu Arg Cys Glu	
<u>g</u> <u>ct</u> <u>cc</u> <u>cg</u> <u>tg</u> <u>tt</u> <u>cc</u> <u>ca</u> <u>g</u> <u>cc</u> <u>ac</u> <u>gt</u> <u>g</u> <u>tc</u> <u>tg</u> <u>g</u> <u>ca</u> <u>t</u> <u>cc</u> <u>ca</u> <u>aa</u> <u>gt</u>	5418
Ala Pro Arg Trp Phe Pro Gln Pro Thr Val Val Trp Ala Ser Gln Val	
EXON 4	
<u>gac</u> <u>cag</u> <u>gga</u> <u>gcc</u> <u>aa</u> <u>c</u> <u>ttc</u> <u>tcg</u> <u>gaa</u> <u>g</u> <u>tc</u> <u>tcc</u> <u>aa</u> <u>t</u> <u>cc</u> <u>g</u> <u>at</u> <u>ttt</u> <u>q</u> <u>ag</u> <u>ct</u> <u>g</u>	5466
Asp Gln Gly Ala Asn Phe Ser Glu Val Ser Asn Thr Ser Phe Glu Leu	
<u>aa</u> <u>ac</u> <u>t</u> <u>ct</u> <u>g</u> <u>ag</u> <u>aa</u> <u>t</u> <u>gt</u> <u>ac</u> <u>at</u> <u>g</u> <u>ag</u> <u>gt</u> <u>t</u> <u>tt</u> <u>gt</u> <u>tc</u> <u>tc</u> <u>ta</u> <u>aa</u> <u>t</u> <u>tt</u>	5514
Asn Ser Glu Asn Val Thr Met Lys Val Val Ser Val Leu Tyr Asn Val	
<u>ac</u> <u>g</u> <u>at</u> <u>tc</u> <u>aa</u> <u>ac</u> <u>ac</u> <u>ca</u> <u>ta</u> <u>cc</u> <u>t</u> <u>gt</u> <u>at</u> <u>g</u> <u>aa</u> <u>aa</u> <u>t</u> <u>ac</u> <u>tt</u> <u>g</u> <u>cc</u> <u>aa</u>	5562
Thr Ile Asn Asn Thr Tyr Ser Cys Met Ile Glu Asn Asp Ile Ala Lys	

FIG. 3E

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 Ser Glu

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 Ile Lys Arg Arg Ser His Leu Gln Leu Ieu Asn Ser Lys Ala Ser Leu

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tcg tga tac aat ggc tga agg aag gtg ttt tag gct tgg tcc atg agt 4298
tca aag aag gca aag atg agc tgt cgg agc agg atg aaa tgt tca gag 4346
gcc gga cag cag tgt ttg ctg atc aag tga tag ttg gca atg cct ctt 4394
tgc ggc tga aaa acg tgc aac tca cag atg ctg gca cct aca aat gtt 4442
ata tca tca ott cta aag gca agg gga atg cta acc ttg agt ata aaa 4490
ctg gag gtgagttact tttggagaga gatTTTAA agcacccaaa gtatTTGAGG 4546
ctaaagatta tgagttgctt atgaaatatg ttgtgaccaa tatcagacaa tcatgctccc 4606
aattctgcta acagctgttt ttgcatttt atggccaaga ctcacaccaa ccccaaaagt 4666
agccaaactat aagagaggcc ttaaaaatct tttgttctgg gttctctctt ctccagaatt 4726
tccagggtgtt ttctctattt aacaaaataa atgttagcctc ttttgtggta gcccctggta 4786
cagctataaa gagcagccata gcctgggaca tttccacgctt aagtagaccc aagcgcaatc 4846
ctgaaatcag tatcttcaga gactcatgac agtgagtgc ttttagaagt tcaaaggaa 4906

ctaaccaaaa cggaaaacaac attgtcctat aaactcatta atnnnnnnnn nnaggaaaaat 4966
gcctaattcc ctaagggata ggcttttta tgaccactga agttccttctt acttggagaa 5026
aattttctat tptaaccata atatacctag aatttaggtc cttggggttt gtgattggcc 5086
aaagggttgt gatgtatagc aatactatga gcaaataattt attcgcacctt ataatacaat 5146
gggctataca caggtggacc tctgaggaag tactgagtgg cttcaatgtt aaaaaaaaaa 5206
aaaaaaaaat gactgcattt ttaaggccta gcgcctcaaa ggctatattt tcttacgtgt 5266
ctgactttgg catctgcctt ttctgacttt tgaccctgca g cct tca gca tgc egg 5322
aag tga atg tgg act ata atg cca got cag aga cct tgc ggt gtg agg 5370
ctc ccc gat ggt tcc ccc agc cca cag tgg tct ggg cat ccc aag ttg 5418
acc agg gag cca act tct cgg aag tct cca ata cca gct ttg agc tga 5466
act ctg aga atg tga cca tga agg ttg tgt ctg tgc tct aca atg tta 5514
cga tca aca aca cat act cct gta tga ttg aaa atg aca ttg cca aag 5562
caa cag ggg ata tca aag tga cag gtgggttctt gcatgccttt gtatggattt 5616
actggggaaa gagtaaaatc taaattaaaa ttaacttca ttaatagata tnnnnnnnnn 5676
ncggctgagt gttcaacttggaa gtggaccaca gaatggattt ttctttgaag aaagagaag 5736
ctctactttt aagccagttt cggagaggtt ggcggaaagggg taaaaaantt gcctgaattt 5796
ggaacaactt ngttctgttt ttcaaggactt tttctttgtt aaccagtcat gtgaagaaga 5856
catccagttt ctcctgtatg accctaaactt tttctctca ctccacagaa tcg gag 5912
atc aaa agg cgg agt cac cta cag ctg cta aac tca aag got tct ctg 5960
tgt gtc tct ttc ttt gcc atc agc tgg gca ctt ctg cct ctc agc 6008
cct tac ctg atg cta aaa taa tgt gcc ttg gcc aca aaa aag cat gca 6056
aag tca ttg tta caa cag gtgagattaa tcacaaatag tgtggatatt 6104
tggttctgtt ggtgtttgtt ctccatatgtt gaggcgata gccttcagca tcagccacag 6164
aaagaaacat ttaatgacac cagggtgtt acattatttc caactgtactt gggaaaggtt 6224
atcatttgtt aggtcagcaa acagcataga cttcattgtt acatccatag gaaaaataac 6284
atacaattca agtattttatg taaattcaaa tacctttnaa gttactcagt catcaatgaa 6344
ggcaaaataaa cttatgaaga gaaaga 6370

<210> 5
<211> 11
<212> PRT
<213> Homo sapiens

<400> 5

Met Ala Ser Leu Gly Gln Ile Leu Phe Trp Ser
 1 5 10

<210> 6
 <211> 22
 <212> PRT
 <213> Homo sapiens

<400> 6
 Ile Ile Ser Ile Ile Ile Leu Ala Gly Ala Ile Ala Leu Ile Ile
 1 5 10 15

Gly Phe Gly Ile Ser Gly
 20

<210> 7
 <211> 116
 <212> PRT
 <213> Homo sapiens

<400> 7
 Arg His Ser Ile Thr Val Thr Val Ala Ser Ala Gly Asn Ile Gly
 1 5 10 15

Glu Asp Gly Ile Leu Ser Cys Thr Phe Glu Pro Asp Ile Lys Leu Ser
 20 25 30

Asp Ile Val Ile Gln Trp Leu Lys Glu Gly Val Leu Gly Leu Val His
 35 40 45

Glu Phe Lys Glu Gly Lys Asp Glu Leu Ser Glu Gln Asp Glu Met Phe
 50 55 60

Arg Gly Arg Thr Ala Val Phe Ala Asp Gln Val Ile Val Gly Asn Ala
 65 70 75 80

Ser Leu Arg Leu Lys Asn Val Gln Leu Thr Asp Ala Gly Thr Tyr Lys
 85 90 95

Cys Tyr Ile Ile Thr Ser Lys Gly Lys Gly Asn Ala Asn Leu Glu Tyr
 100 105 110

Lys Thr Gly Ala
 115

<210> 8
 <211> 93
 <212> PRT
 <213> Homo sapiens

<400> 8
 Phe Ser Met Pro Glu Val Asn Val Asp Tyr Asn Ala Ser Ser Glu Thr
 1 5 10 15

Leu Arg Cys Glu Ala Pro Arg Trp Phe Pro Gln Pro Thr Val Val Trp
 20 25 30

Ala Ser Gln Val Asp Gln Gly Ala Asn Phe Ser Glu Val Ser Asn Thr

35	40	45
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Ser Phe Glu Leu Asn Ser Glu Asn Val Thr Met Lys Val Val Ser Val
 50 55 60

Leu Tyr Asn Val Thr Ile Asn Asn Thr Tyr Ser Cys Met Ile Glu Asn
 65 70 75 80

Asp Ile Ala Lys Ala Thr Gly Asp Ile Lys Val Thr Glu
 85 90

<210> 9

<211> 40

<212> PRT

<213> Homo sapiens

<400> 9

Ser Glu Ile Lys Arg Arg Ser His Leu Gln Leu Leu Asn Ser Lys Ala
 1 5 10 15

Ser Leu Cys Val Ser Ser Phe Phe Ala Ile Ser Trp Ala Leu Leu Pro
 20 25 30

Leu Ser Pro Tyr Leu Met Leu Lys
 35 40

<210> 10

<211> 288

<212> PRT

<213> Homo sapiens

<400> 10

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
 1 5 10 15

Ieu Asn Phe Phe Gln Leu Leu Val Ieu Ala Gly Leu Ser His Phe Cys
 20 25 30

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu
 35 40 45

Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
 50 55 60

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
 65 70 75 80

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
 85 90 95

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
 100 105 110

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
 115 120 125

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
 130 135 140

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
 145 150 155 160
 Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
 165 170 175
 Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
 180 185 190
 Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
 195 200 205
 Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
 210 215 220
 Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
 225 230 235 240
 Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
 245 250 255
 Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala Pro Arg Cys Arg
 260 265 270
 Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
 275 280 285

<210> 11
 <211> 323
 <212> PRT
 <213> Homo sapiens

<400> 11
 Met Gly Leu Ser Asn Ile Leu Phe Val Met Ala Phe Leu Leu Ser Gly
 1 5 10 15
 Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe Asn Glu Thr Ala Asp Leu
 20 25 30
 Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu Ser Glu Leu Val
 35 40 45
 Val Phe Trp Gln Asp Gln Glu Asn Leu Val Leu Asn Glu Val Tyr Leu
 50 55 60
 Gly Lys Glu Lys Phe Asp Ser Val His Ser Lys Tyr Met Gly Arg Thr
 65 70 75 80
 Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg Leu His Asn Leu Gln Ile
 85 90 95
 Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile His His Lys Lys Pro Thr
 100 105 110
 Gly Met Ile Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala
 115 120 125

Asn Phe Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ile Thr Glu Asn
 130 135 140
 Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile His Gly Tyr Pro Glu Pro
 145 150 155 160
 Lys Lys Met Ser Val Leu Leu Arg Thr Lys Asn Ser Thr Ile Glu Tyr
 165 170 175
 Asp Gly Ile Met Gln Lys Ser Gln Asp Asn Val Thr Glu Leu Tyr Asp
 180 185 190
 Val Ser Ile Ser Leu Ser Val Ser Phe Pro Asp Val Thr Ser Asn Met
 195 200 205
 Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys Thr Arg Leu Leu Ser Ser
 210 215 220
 Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln Pro Pro Pro Asp His Ile
 225 230 235 240
 Pro Trp Ile Thr Ala Val Leu Pro Thr Val Ile Ile Cys Val Met Val
 245 250 255
 Phe Cys Leu Ile Leu Trp Lys Trp Lys Lys Lys Arg Pro Arg Asn
 260 265 270
 Ser Tyr Lys Cys Gly Thr Asn Thr Met Glu Arg Glu Glu Ser Glu Gln
 275 280 285
 Thr Lys Lys Arg Glu Lys Ile His Ile Pro Glu Arg Ser Asp Glu Ala
 290 295 300
 Gln Arg Val Phe Lys Ser Ser Lys Thr Ser Ser Cys Asp Lys Ser Asp
 305 310 315 320
 Thr Cys Phe

<210> 12
 <211> 290
 <212> PRT
 <213> Homo sapiens

<400> 12
 Met Arg Ile Phe Ala Val Phe Met Thr Tyr Trp His Leu Leu
 1 5 10 15
 Asn Ala Phe Thr Val Thr Val Pro Asp Lys Leu Tyr Val Val Glu Tyr
 20 25 30
 Gly Ser Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu
 35 40 45
 Asp Leu Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile
 50 55 60
 Ile Gln Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser
 65 70 75 80

Tyr Arg Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn
 85 90 95

Ala Ala Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr
 100 105 110

Arg Cys Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val
 115 120 125

Lys Val Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val
 130 135 140

Asp Pro Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr
 145 150 155 160

Pro Lys Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser
 165 170 175

Gly Lys Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn
 180 185 190

Val Thr Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr
 195 200 205

Cys Thr Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu
 210 215 220

Val Ile Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr His
 225 230 235 240

Leu Val Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr
 245 250 255

Phe Ile Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys
 260 265 270

Gly Ile Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu
 275 280 285

Glu Thr
 290

<210> 13
<211> 302
<212> PRT
<213> Homo sapiens

<400> 13
Met Arg Leu Gly Ser Pro Gly Leu Leu Phe Leu Leu Phe Ser Ser Leu
 1 5 10 15

Arg Ala Asp Thr Gln Glu Lys Glu Val Arg Ala Met Val Gly Ser Asp
 20 25 30

Val Glu Leu Ser Cys Ala Cys Pro Glu Gly Ser Arg Phe Asp Leu Asn
 35 40 45

Asp Val Tyr Val Tyr Trp Gln Thr Ser Glu Ser Lys Thr Val Val Thr
 50 55 60

Tyr His Ile Pro Gln Asn Ser Ser Leu Glu Asn Val Asp Ser Arg Tyr
 65 70 75 80
 Arg Asn Arg Ala Leu Met Ser Pro Ala Gly Met Leu Arg Gly Asp Phe
 85 90 95
 Ser Leu Arg Leu Phe Asn Val Thr Pro Gln Asp Glu Gln Lys Phe His
 100 105 110
 Cys Leu Val Leu Ser Gln Ser Leu Gly Phe Gln Glu Val Leu Ser Val
 115 120 125
 Glu Val Thr Leu His Val Ala Ala Asn Phe Ser Val Pro Val Val Ser
 130 135 140
 Ala Pro His Ser Pro Ser Gln Asp Glu Leu Thr Phe Thr Cys Thr Ser
 145 150 155 160
 Ile Asn Gly Tyr Pro Arg Pro Asn Val Tyr Trp Ile Asn Lys Thr Asp
 165 170 175
 Asn Ser Leu Leu Asp Gln Ala Leu Gln Asn Asp Thr Val Phe Leu Asn
 180 185 190
 Met Arg Gly Leu Tyr Asp Val Val Ser Val Leu Arg Ile Ala Arg Thr
 195 200 205
 Pro Ser Val Asn Ile Gly Cys Cys Ile Glu Asn Val Leu Leu Gln Gln
 210 215 220
 Asn Leu Thr Val Gly Ser Gln Thr Gly Asn Asp Ile Gly Glu Arg Asp
 225 230 235 240
 Lys Ile Thr Glu Asn Pro Val Ser Thr Gly Glu Lys Asn Ala Ala Thr
 245 250 255
 Trp Ser Ile Leu Ala Val Leu Cys Leu Leu Val Val Ala Val Ala
 260 265 270
 Ile Gly Trp Val Cys Arg Asp Arg Cys Leu Gln His Ser Tyr Ala Gly
 275 280 285
 Ala Trp Ala Val Ser Pro Glu Thr Glu Leu Thr Gly His Val
 290 295 300

<210> 14
<211> 316
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> (233)
<223> "Xaa" can be any naturally occurring amino acid

<400> 14
Met Leu Arg Arg Arg Gly Ser Pro Gly Met Gly Val His Val Gly Ala
 1 5 10 15

Ala Leu Gly Ala Leu Trp Phe Cys Leu Thr Gly Ala Leu Glu Val Gln

20

25

30

Val Pro Glu Asp Pro Val Val Ala Leu Val Gly Thr Asp Ala Thr Leu
 35 40 45

Cys Cys Ser Phe Ser Pro Glu Pro Gly Phe Ser Leu Ala Gln Leu Asn
 50 55 60

Leu Ile Trp Gln Leu Thr Asp Thr Lys Gln Leu Val His Ser Phe Ala
 65 70 75 80

Glu Gly Gln Asp Gln Gly Ser Ala Tyr Ala Asn Arg Thr Ala Leu Phe
 85 90 95

Pro Asp Leu Leu Ala Gln Gly Asn Ala Ser Leu Arg Leu Gln Arg Val
 100 105 110

Arg Val Ala Asp Glu Gly Ser Phe Thr Cys Phe Val Ser Ile Arg Asp
 115 120 125

Phe Gly Ser Ala Ala Val Ser Leu Gln Val Ala Ala Pro Tyr Ser Lys
 130 135 140

Pro Ser Met Thr Leu Glu Pro Asn Lys Asp Leu Arg Pro Gly Asp Thr
 145 150 155 160

Val Thr Ile Thr Cys Ser Ser Tyr Gln Gly Tyr Pro Glu Ala Glu Val
 165 170 175

Phe Trp Gln Asp Gly Gln Gly Val Pro Leu Thr Gly Asn Val Thr Thr
 180 185 190

Ser Gln Met Ala Asn Glu Gln Gly Leu Phe Asp Val His Ser Val Leu
 195 200 205

Arg Val Val Leu Gly Ala Asn Gly Thr Tyr Ser Cys Leu Val Arg Asn
 210 215 220

Pro Val Leu Gln Gln Asp Ala His Xaa Ser Val Thr Ile Thr Gly Gln
 225 230 235 240

Pro Met Thr Phe Pro Pro Glu Ala Leu Trp Val Thr Val Gly Leu Ser
 245 250 255

Val Cys Leu Ile Ala Leu Leu Val Ala Leu Ala Phe Val Cys Trp Arg
 260 265 270

Lys Ile Lys Gln Ser Cys Glu Glu Glu Asn Ala Gly Ala Glu Asp Gln
 275 280 285

Asp Gly Glu Gly Glu Ser Lys Thr Ala Leu Gln Pro Leu Lys His
 290 295 300

Ser Asp Ser Lys Glu Asp Asp Gly Gln Glu Ile Ala
 305 310 315

<210> 15

<211> 276

<212> PRT

<213> Homo sapiens

<400> 15
 Met Glu Ser Ala Ala Ala Leu His Phe Ser Arg Pro Ala Ser Leu Leu
 1 5 10 15

Leu Leu Leu Leu Ser Leu Cys Ala Leu Val Ser Ala Gln Phe Ile Val
 20 25 30

Val Gly Pro Thr Asp Pro Ile Leu Ala Thr Val Gly Glu Asn Thr Thr
 35 40 45

Leu Arg Cys His Leu Ser Pro Glu Lys Asn Ala Glu Asp Met Glu Val
 50 55 60

Arg Trp Phe Arg Ser Gln Phe Ser Pro Ala Val Phe Val Tyr Lys Gly
 65 70 75 80

Gly Arg Glu Arg Thr Glu Glu Gln Met Glu Glu Tyr Arg Gly Arg Thr
 85 90 95

Thr Phe Val Ser Lys Asp Ile Ser Arg Gly Ser Val Ala Leu Val Ile
 100 105 110

His Asn Ile Thr Ala Gln Glu Asn Gly Thr Tyr Arg Cys Tyr Phe Gln
 115 120 125

Glu Gly Arg Ser Tyr Asp Glu Ala Ile Leu His Leu Val Val Ala Gly
 130 135 140

Leu Gly Ser Lys Pro Leu Ile Ser Met Arg Gly His Glu Asp Gly Gly
 145 150 155 160

Ile Arg Leu Glu Cys Ile Ser Arg Gly Trp Tyr Pro Lys Pro Leu Thr
 165 170 175

Val Trp Arg Asp Pro Tyr Gly Gly Val Ala Pro Ala Leu Lys Glu Val
 180 185 190

Ser Met Pro Asp Ala Asp Gly Leu Phe Met Val Thr Thr Ala Val Ile
 195 200 205

Ile Arg Asp Lys Ser Val Arg Asn Met Ser Cys Ser Ile Asn Asn Thr
 210 215 220

Leu Leu Gly Gln Lys Lys Glu Ser Val Ile Phe Ile Pro Glu Ser Phe
 225 230 235 240

Met Pro Ser Val Ser Pro Cys Ala Val Ala Leu Pro Ile Ile Val Val
 245 250 255

Ile Leu Met Ile Pro Ile Ala Val Cys Ile Tyr Trp Ile Asn Lys Leu
 260 265 270

Gln Lys Glu Lys
 275

<210> 16
 <211> 523
 <212> PRT
 <213> Homo sapiens

<400> 16
Met Glu Pro Ala Ala Ala Leu His Phe Ser Leu Pro Ala Ser Leu Leu
1 5 10 15
Leu Leu Leu Leu Leu Leu Leu Ser Leu Cys Ala Leu Val Ser Ala
20 25 30
Gln Phe Thr Val Val Gly Pro Ala Asn Pro Ile Leu Ala Met Val Gly
35 40 45
Glu Asn Thr Thr Leu Arg Cys His Leu Ser Pro Glu Lys Asn Ala Glu
50 55 60
Asp Met Glu Val Arg Trp Phe Arg Ser Gln Phe Ser Pro Ala Val Phe
65 70 75 80
Val Tyr Lys Gly Gly Arg Glu Arg Thr Glu Glu Gln Met Glu Glu Tyr
85 90 95
Arg Gly Arg Ile Thr Phe Val Ser Lys Asp Ile Asn Arg Gly Ser Val
100 105 110
Ala Leu Val Ile His Asn Val Thr Ala Gln Glu Asn Gly Ile Tyr Arg
115 120 125
Cys Tyr Phe Gln Glu Gly Arg Ser Tyr Asp Glu Ala Ile Leu Arg Leu
130 135 140
Val Val Ala Gly Leu Gly Ser Lys Pro Leu Ile Glu Ile Lys Ala Gln
145 150 155 160
Glu Asp Gly Ser Ile Trp Leu Glu Cys Ile Ser Gly Gly Trp Tyr Pro
165 170 175
Glu Pro Leu Thr Val Trp Arg Asp Pro Tyr Gly Glu Val Val Pro Ala
180 185 190
Leu Lys Glu Val Ser Ile Ala Asp Ala Asp Gly Leu Phe Met Val Thr
195 200 205
Thr Ala Val Ile Ile Arg Asp Lys Tyr Val Arg Asn Val Ser Cys Ser
210 215 220
Val Asn Asn Thr Leu Leu Gly Gln Glu Lys Glu Thr Val Ile Phe Ile
225 230 235 240
Pro Glu Ser Phe Met Pro Ser Ala Ser Pro Trp Met Val Ala Leu Ala
245 250 255
Val Ile Leu Thr Ala Ser Pro Trp Met Val Ser Met Thr Val Ile Leu
260 265 270
Ala Val Phe Ile Ile Phe Met Ala Val Ser Ile Cys Cys Ile Lys Lys
275 280 285
Leu Gln Arg Glu Lys Lys Ile Leu Ser Gly Glu Lys Lys Val Glu Gln
290 295 300
Glu Glu Lys Glu Ile Ala Gln Gln Leu Gln Glu Glu Leu Arg Trp Arg
305 310 315 320

Arg Thr Phe Leu His Ala Ala Asp Val Val Leu Asp Pro Asp Thr Ala
 325 330 335
 His Pro Glu Leu Phe Leu Ser Glu Asp Arg Arg Ser Val Arg Arg Gly
 340 345 350
 Pro Tyr Arg Gln Arg Val Pro Asp Asn Pro Glu Arg Phe Asp Ser Gln
 355 360 365
 Pro Cys Val Leu Gly Trp Glu Ser Phe Ala Ser Gly Lys His Tyr Trp
 370 375 380
 Glu Val Glu Val Glu Asn Val Met Val Trp Thr Val Gly Val Cys Arg
 385 390 395 400
 His Ser Val Glu Arg Lys Gly Glu Val Leu Leu Ile Pro Gln Asn Gly
 405 410 415
 Phe Trp Thr Leu Glu Met Phe Gly Asn Gln Tyr Arg Ala Leu Ser Ser
 420 425 430
 Pro Glu Arg Ile Leu Pro Leu Lys Glu Ser Leu Cys Arg Val Gly Val
 435 440 445
 Phe Leu Asp Tyr Glu Ala Gly Asp Val Ser Phe Tyr Asn Met Arg Asp
 450 455 460
 Arg Ser His Ile Tyr Thr Cys Pro Arg Ser Ala Phe Thr Val Pro Val
 465 470 475 480
 Arg Pro Phe Phe Arg Leu Gly Ser Asp Asp Ser Pro Ile Phe Ile Cys
 485 490 495
 Pro Ala Leu Thr Gly Ala Ser Gly Val Met Val Pro Glu Glu Gly Leu
 500 505 510
 Lys Leu His Arg Val Gly Thr His Gln Ser Leu
 515 520

<210> 17
 <211> 263
 <212> PRT
 <213> Homo sapiens

<400> 17
 Phe His Val Ser Leu Leu Leu Val Gln Leu Leu Thr Pro Cys Ser Ala
 1 5 10 15
 Gln Phe Ser Val Leu Gly Pro Ser Gly Pro Ile Leu Ala Met Val Gly
 20 25 30
 Glu Asp Ala Asp Leu Pro Cys His Leu Phe Pro Thr Met Ser Ala Glu
 35 40 45
 Thr Met Glu Leu Lys Trp Val Ser Ser Ser Leu Arg Gln Val Val Asn
 50 55 60
 Val Tyr Ala Asp Gly Lys Glu Val Glu Asp Arg Gln Ser Ala Pro Tyr
 65 70 75 80

Arg Gly Arg Thr Ser Ile Leu Arg Asp Gly Ile Thr Ala Gly Lys Ala
 85 90 95
 Ala Leu Arg Ile His Asn Val Thr Ala Ser Asp Ser Gly Lys Tyr Leu
 100 105 110
 Cys Tyr Phe Gln Asp Gly Asp Phe Tyr Glu Lys Ala Leu Val Glu Leu
 115 120 125
 Lys Val Ala Ala Leu Gly Ser Asn Leu His Val Glu Val Lys Gly Tyr
 130 135 140
 Glu Asp Gly Gly Ile His Leu Glu Cys Arg Ser Thr Gly Trp Tyr Pro
 145 150 155 160
 Gln Pro Gln Ile Gln Trp Ser Asn Ala Lys Gly Glu Asn Ile Pro Ala
 165 170 175
 Val Glu Ala Pro Val Val Ala Asp Gly Val Gly Leu Tyr Glu Val Ala
 180 185 190
 Ala Ser Val Ile Met Arg Gly Gly Ser Gly Glu Gly Val Ser Cys Ile
 195 200 205
 Ile Arg Asn Ser Leu Leu Gly Leu Glu Lys Thr Ala Ser Ile Ser Ile
 210 215 220
 Ala Asp Pro Phe Phe Arg Ser Ala Gln Pro Trp Ile Ala Ala Leu Ala
 225 230 235 240
 Gly Thr Leu Pro Ile Leu Leu Leu Ala Gly Ala Ser Tyr Phe
 245 250 255
 Leu Trp Arg Gln Gln Lys Glu
 260

<210> 18
 <211> 584
 <212> PRT
 <213> Homo sapiens

<400> 18
 Met Lys Met Ala Ser Ser Leu Ala Phe Leu Leu Leu Asn Phe His Val
 1 5 10 15
 Ser Leu Phe Leu Val Gln Leu Leu Thr Pro Cys Ser Ala Gln Phe Ser
 20 25 30
 Val Leu Gly Pro Ser Gly Pro Ile Leu Ala Met Val Gly Glu Asp Ala
 35 40 45
 Asp Leu Pro Cys His Leu Phe Pro Thr Met Ser Ala Glu Thr Met Glu
 50 55 60
 Leu Arg Trp Val Ser Ser Leu Arg Gln Val Val Asn Val Tyr Ala,
 65 70 75 80
 Asp Gly Lys Glu Val Glu Asp Arg Gln Ser Ala Pro Tyr Arg Gly Arg
 85 90 95

Thr Ser Ile Leu Arg Asp Gly Ile Thr Ala Gly Lys Ala Ala Leu Arg
 100 105 110
 Ile His Asn Val Thr Ala Ser Asp Ser Gly Lys Tyr Leu Cys Tyr Phe
 115 120 125
 Gln Asp Gly Asp Phe Tyr Glu Lys Ala Leu Val Glu Leu Lys Val Ala
 130 135 140
 Ala Leu Gly Ser Asp Leu His Ile Glu Val Lys Gly Tyr Glu Asp Gly
 145 150 155 160
 Gly Ile His Leu Glu Cys Arg Ser Thr Gly Trp Tyr Pro Gln Pro Gln
 165 170 175
 Ile Lys Trp Ser Asp Thr Lys Gly Glu Asn Ile Pro Ala Val Glu Ala
 180 185 190
 Pro Val Val Ala Asp Gly Val Gly Leu Tyr Ala Val Ala Ala Ser Val
 195 200 205
 Ile Met Arg Gly Ser Ser Gly Gly Val Ser Cys Ile Ile Arg Asn
 210 215 220
 Ser Leu Leu Gly Leu Glu Lys Thr Ala Ser Ile Ser Ile Ala Asp Pro
 225 230 235 240
 Phe Phe Arg Ser Ala Gln Pro Trp Ile Ala Ala Leu Ala Gly Thr Leu
 245 250 255
 Pro Ile Ser Leu Leu Leu Ala Gly Ala Ser Tyr Phe Leu Trp Arg
 260 265 270
 Gln Gln Lys Glu Lys Ile Ala Leu Ser Arg Glu Thr Glu Arg Glu Arg
 275 280 285
 Glu Met Lys Glu Met Gly Tyr Ala Ala Thr Glu Gln Glu Ile Ser Leu
 290 295 300
 Arg Glu Lys Leu Gln Glu Leu Lys Trp Arg Lys Ile Gln Tyr Met
 305 310 315 320
 Ala Arg Gly Glu Lys Ser Leu Ala Tyr His Glu Trp Lys Met Ala Leu
 325 330 335
 Phe Lys Pro Ala Asp Val Ile Leu Asp Pro Asp Thr Ala Asn Ala Ile
 340 345 350
 Leu Leu Val Ser Glu Asp Gln Arg Ser Val Gln Arg Ala Glu Glu Pro
 355 360 365
 Arg Asp Leu Pro Asp Asn Pro Glu Arg Phe Glu Trp Arg Tyr Cys Val
 370 375 380
 Leu Gly Cys Glu Asn Phe Thr Ser Gly Arg His Tyr Trp Glu Val Glu
 385 390 395 400
 Val Gly Asp Arg Lys Glu Trp His Ile Gly Val Cys Ser Lys Asn Val
 405 410 415

Glu Arg Lys Lys Gly Trp Val Lys Met Thr Pro Glu Asn Gly Tyr Trp
 420 425 430
 Thr Met Gly Leu Thr Asp Gly Asn Lys Tyr Arg Ala Leu Thr Glu Pro
 435 440 445
 Arg Thr Asn Leu Lys Leu Pro Glu Pro Pro Arg Lys Val Gly Ile Phe
 450 455 460
 Leu Asp Tyr Glu Thr Gly Glu Ile Ser Phe Tyr Asn Ala Thr Asp Gly
 465 470 475 480
 Ser His Ile Tyr Thr Phe Pro His Ala Ser Phe Ser Glu Pro Leu Tyr
 485 490 495
 Pro Val Phe Arg Ile Leu Thr Leu Glu Pro Thr Ala Leu Thr Ile Cys
 500 505 510
 Pro Ile Pro Lys Glu Val Glu Ser Ser Pro Asp Pro Asp Leu Val Pro
 515 520 525
 Asp His Ser Leu Glu Thr Pro Leu Thr Pro Gly Leu Ala Asn Glu Ser
 530 535 540
 Gly Glu Pro Gln Ala Glu Val Thr Ser Leu Leu Leu Pro Ala His Pro
 545 550 555 560
 Gly Ala Glu Val Ser Pro Ser Ala Thr Thr Asn Gln Asn His Lys Leu
 565 570 575
 Gln Ala Arg Thr Glu Ala Leu Tyr
 580

<210> 19
 <211> 526
 <212> PRT
 <213> Homo sapiens

<400> 19
 Met Ala Val Phe Pro Ser Ser Gly Leu Pro Arg Cys Leu Leu Thr Leu
 1 5 10 15
 Ile Leu Leu Gln Leu Pro Lys Leu Asp Ser Ala Pro Phe Asp Val Ile
 20 25 30
 Gly Pro Pro Glu Pro Ile Leu Ala Val Val Gly Glu Asp Ala Glu Leu
 35 40 45
 Pro Cys Arg Leu Ser Pro Asn Ala Ser Ala Glu His Leu Glu Leu Arg
 50 55 60
 Trp Phe Arg Lys Lys Val Ser Pro Ala Val Leu Val His Arg Asp Gly
 65 70 75 80
 Arg Glu Gln Glu Ala Glu Gln Met Pro Glu Tyr Arg Gly Arg Ala Thr
 85 90 95
 Leu Val Gln Asp Gly Ile Ala Lys Gly Arg Val Ala Leu Arg Ile Arg
 100 105 110

Gly Val Arg Val Ser Asp Asp Gly Glu Tyr Thr Cys Phe Phe Arg Glu
 115 120 125
 Asp Gly Ser Tyr Glu Glu Ala Leu Val His Leu Lys Val Ala Ala Leu
 130 135 140
 Gly Ser Asp Pro His Ile Ser Met Gln Val Gln Glu Asn Gly Glu Ile
 145 150 155 160
 Cys Leu Glu Cys Thr Ser Val Gly Trp Tyr Pro Glu Pro Gln Val Gln
 165 170 175
 Trp Arg Thr Ser Lys Gly Glu Lys Phe Pro Ser Thr Ser Glu Ser Arg
 180 185 190
 Asn Pro Asp Glu Glu Gly Leu Phe Thr Val Ala Ala Ser Val Ile Ile
 195 200 205
 Arg Asp Thr Ser Thr Lys Asn Val Ser Cys Tyr Ile Gln Asn Leu Leu
 210 215 220
 Leu Gly Gln Glu Lys Lys Val Glu Ile Ser Ile Pro Ala Ser Ser Leu
 225 230 235 240
 Pro Arg Leu Thr Pro Trp Ile Val Ala Val Ala Val Ile Leu Met Val
 245 250 255
 Leu Gly Leu Leu Thr Ile Gly Ser Ile Phe Phe Thr Trp Arg Leu Tyr
 260 265 270
 Asn Glu Arg Pro Arg Glu Arg Arg Asn Glu Phe Ser Ser Lys Glu Arg
 275 280 285
 Leu Leu Glu Leu Lys Trp Lys Lys Ala Thr Leu His Ala Val Asp
 290 295 300
 Val Thr Leu Asp Pro Asp Thr Ala His Pro His Leu Phe Leu Tyr Glu
 305 310 315 320
 Asp Ser Lys Ser Val Arg Leu Glu Asp Ser Arg Gln Lys Leu Pro Glu
 325 330 335
 Lys Thr Glu Arg Phe Asp Ser Trp Pro Cys Val Leu Gly Arg Glu Thr
 340 345 350
 Phe Thr Ser Gly Arg His Tyr Trp Glu Val Glu Val Gly Asp Arg Thr
 355 360 365
 Asp Trp Ala Ile Gly Val Cys Arg Glu Asn Val Met Lys Lys Gly Phe
 370 375 380
 Asp Pro Met Thr Pro Glu Asn Gly Phe Trp Ala Val Glu Leu Tyr Gly
 385 390 395 400
 Asn Gly Tyr Trp Ala Leu Thr Pro Leu Arg Thr Pro Leu Pro Leu Ala
 405 410 415
 Gly Pro Pro Arg Arg Val Gly Ile Phe Leu Asp Tyr Glu Ser Gly Asp
 420 425 430
 Ile Ser Phe Tyr Asn Met Asn Asp Gly Ser Asp Ile Tyr Thr Phe Ser

435	440	445
Asn Val Thr Phe Ser Gly Pro Leu Arg Pro Phe Phe Cys Leu Trp Ser		
450	455	460
Ser Gly Lys Lys Pro Leu Thr Ile Cys Pro Ile Ala Asp Gly Pro Glu		
465	470	475
Arg Val Thr Val Ile Ala Asn Ala Gln Asp Leu Ser Lys Glu Ile Pro		
485	490	495
Leu Ser Pro Met Gly Glu Glu Ser Ala Pro Arg Asp Ala Asp Thr Leu		
500	505	510
His Ser Lys Leu Ile Pro Thr Gln Pro Ser Gln Gly Ala Pro		
515	520	525

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Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
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domain derived from HIV tat protein

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Oligonucleotide 2434-29

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<223> Description of Artificial Sequence:
Oligonucleotide 2434-34

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19

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